GUAYULE

Natural Rubber

A Technical Publication with Emphasis on Recent Findings

Guayule Administrative
Management Committee
and
USDA Cooperative
State Research Service

J. Wayne Whitworth & Emily E. Whitehead, editors

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Table of Contents

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Preface

Collected within these pages is a body of work representing the results of years of research undertaken by individuals dedicated to furthering the understanding of a desert plant that may one day be able to supply a domestic source of natural rubber. Those individuals and their collective efforts in studying this plant, guayule (*Parthenium argentatum* Gray), have made this book possible.

The Guayule Administrative Management Committee (GAMC) commissioned this publication as a definitive source of this work, which represents the state-of-the-art of guayule as we know it today. The book covers many aspects of guayule—from biochemical evolution and plant genetics to the latest advances in coproducts research.

Partial funding for development of the publication was provided by the U.S. Department of Agriculture (USDA), Cooperative State Research Service (CSRS).

Guayule Natural Rubber was produced by the Office of Arid Lands Studies, College of Agriculture, The University of Arizona.

Foreword

Guayule, *Parthenium argentatum* Gray, is one of the remarkable plants native to the desert regions of southwestern Texas and northern Mexico. This book documents the results of the research and development activities associated with the third major effort in this century to develop guayule as a domestic source of natural rubber for the United States of America.

I first encountered the obscure and strange-sounding word "guayule" (pronounced wy-oo-lee) and the plant it represents more than thirteen years ago. In the spring of 1976, William P. Miller of the U.S. Bureau of Indian Affairs came to see me and gave me a quick briefing on the history of guayule in North America. I was then in charge of a program at the National Science Foundation (NSF) that supported research on renewable biological materials that could replace petroleum as sources of energy and chemical feedstock. Guayule appeared to be just the right type of resource for support under the auspices of the NSF program. Research proposals were soon received and the first grants were awarded shortly thereafter, in the late summer of 1976.

But a serious coordinated effort on guayule did not really begin in earnest until 1979 after the enactment of PL 95-592, the Native Latex Commercialization and Economic Development Act of 1978 (or Native Latex Act), when the U.S. Department of Agriculture (USDA) took over major responsibility for implementing the provisions of the act. Thus, we may say that this recent national program on guayule has been in existence for about 12 years. Twelve years is a long time in the life of a program. Millions of taxpayers' dollars and hundreds of scientific man years have been spent to determine the potential of guayule as a domestic, commercial source of natural rubber. What have we learned from these expenditures? Here, in this volume, we have the results of an attempt of the guayule community to answer this very question.

Twelve years is also a long time in the life of the individuals involved. During this period we have seen many talented guayule enthusiasts, or "guayuleros," taking up the challenge and continuing to actively contribute to various aspects of this enterprise. Their voices are heard in this publication. But a number of individuals who played key roles in the early years have since moved on to well-deserved retirement or to bigger and better things. They have taught me a good deal about the science, economics, and politics of guayule. Let me, therefore, take this opportunity to commend them to the contributors and readers of this book in appreciation of their services to the guayule community.

I shall start off with Bill Miller whose commitment and zeal did much to energize interest in guayule among government funding agencies in the early days. Next is Reed Rollins who ably chaired the Panel on Guayule convened by the National Academy of Sciences (NAS), the report of which was a major factor in facilitating the passage of the Guayule Act of 1978. However, the success of the NAS report also owes much to the initiative and hard work of the staff director, Noel Vietmeyer, whose enthusiasm for underexploited plants remains unabated today. Another old-time guayulero who actively participated in the new effort, but who has now retired, is Hewitt Tysdal, whose knowledge and experience were most helpful in setting the course of the plant breeding program. Finally, I want to mention two pioneers who were the first researchers to begin field studies on guayule in this era: George Hansen, formerly of the Los Angeles County Arboretum, and David Rubis, formerly of the University of Arizona. The collections they have made and the seeds they have sown and harvested are being used widely by guayuleros in the far corners of the earth.

What then will be the outcome of this, the third major effort on guayule in the United States in this century? Time only will tell. Whatever it is, this compilation will remain an authoritative record of the state-of-the-art on guayule that will be consulted by researchers, policy-makers, and entrepreneurs for years to come.

H.T. Huang
National Science Foundation

Introduction

Natural rubber is one of the few commodities truly essential for the smooth functioning of a modern, industrial economy. Although many plants are known to contain rubber, only two have been utilized as sources of rubber on a commercial scale. One is the rubber tree, *Hevea brasiliensis* (A. Juss.) Muell.-Arg., a native of the Amazon forest, but now grown as a crop in the plantations of Southeast Asia. The other is guayule, *Parthenium argentatum* Gray, an unobtrusive shrub resembling sagebrush that inhabits north-central Mexico and the Big Bend region of southwestern Texas. While today most think of the rubber tree as the only source of natural rubber, it was not always so. At the turn of the present century wild guayule stands in Mexico actually accounted for a substantial share of the rubber for the tires of the automobiles of North America. Reckless exploitation, however, soon exhausted the natural supply and the boom in the desert ended in a bust.[1]

Since then three attempts have been made in the United States to grow guayule as a cultivated crop. The first was a private undertaking in the 1920s by the Continental Rubber Company in Arizona and California, but the effort stumbled with the onset of the Great Depression. The second was the Emergency Rubber Project of World War II. In its heyday it involved 1,000 scientists and technicians, a work force of 9,000 laborers, and 32,000 acres under cultivation at 13 sites in 3 states. But the massive effort collapsed when the war ended and *Hevea* rubber again became available from Southeast Asia.

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[1] National Academy of Sciences. 1977. *Guayule: An alternative source of natural rubber.* 80 pp.

The third, and still ongoing, effort is the raison d'être for this book. The quadrupling of the price of crude oil in the early 1970s prompted a new surge of interest in the United States in all types of domestic, renewable sources of energy, industrial materials, and chemical feedstocks. Among the materials looked at was natural rubber, about 800,000 tons of which are imported from Southeast Asia every year. Guayule was dusted off the shelf and reexamined as a potential domestic source of natural rubber. The Mexicans were the first to translate this interest into action. They built a pilot plant at Saltillo, Province, which had begun to extract rubber from native stands of guayule by early 1975.

Two events occurred in the year 1975 that represent a turning point in the story of the new guayule program. The first was an international conference at the University of Arizona, Tucson, involving old-time guayuleros and new converts to the cause. The proceedings were published as a special report soon after the conference.[2] The second event was the establishment of an ad hoc Panel on Guayule by the National Research Council (NRC) of the National Academy of Sciences (NAS) to consider the potential of guayule as a domestic source of natural rubber for the United States and to make specific recommendations for a plan of action. Under the chairmanship of Reed Rollins of Harvard, the panel first met in Tucson after the international conference and its final report entitled *Guayule: An Alternative Source of Natural Rubber* was published eighteen months later in the fall of 1977.[1]

These two events galvanized the guayuleros and their sympathizers into action, which took two forms. The first was to encourage researchers to submit grant proposals for specific projects on guayule to funding agencies such as the National Science Foundation (NSF), which at that time was in a position to support a certain amount of research related to renewable

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[2] McGinnies. W.G., and E.F. Haase, eds. 1975. *An international conference on the utilization of guayule*, Tucson, Arizona, November 17-19, 1975. Office of Arid Lands Studies, University of Arizona, Tucson. 176 pp.

resources such as guayule. The applications received sympathetic attention. The first grant was awarded in the fall of 1976 to David Rubis, University of Arizona, in collaboration with Reed Rollins, Harvard University, for seed collection and study. Additional awards soon followed. It was recognized, however, that this was merely an interim measure since the development phases of the work were incompatible with the basic mission of the NSF. To achieve a larger and more sustained source of support the guayuleros lobbied Congress to enact a bill that would provide funds for a national program to develop guayule as a domestic source of natural rubber for the United States. They won the support of Congressman George Brown of California, and Senator Pete Domenici of New Mexico. Each introduced a bill that incorporated many of the recommendations of the NAS panel. The Senate and House versions were reconciled and passed in December 1978 as PL 95-592, the Native Latex Commercialization and Economic Development Act of 1978, more commonly known as the Native Latex Act. The act directs the Secretary of Agriculture to "conduct, sponsor, promote, and coordinate basic and applied research, technology development, and technology transfer leading to effective and economical methods for large-scale culturing of plantations and the extraction of latex from *Parthenium*." It also directs the Secretary of Commerce to conduct " . . . demonstration projects to test and demonstrate the economic feasibility of the manufacturing and commercialization of natural rubber from *Parthenium* or other hydrocarbon-containing plants." The act established a joint commission to plan and oversee the program and authorized the expenditure of $30 million in four years to support the research, development, and technology transfer activities specified. The commission consisted of eight members, three from the Department of Agriculture, three from the Department of Commerce, and one each from the NSF and the Department of the Interior. The act was later reauthorized as PL 98-284, the Critical Agricultural Materials Act of 1984. The joint commission was expanded to include a representative each from the Department of State, the Department of Defense, and the Federal Emergency Management Agency.

The commission began its existence in an atmosphere of optimism and promise in early 1979. A staff group was established to assist the commission in implementing the provisions of the act. It was chaired by a representative of the USDA who effectively served as the program manager of the national effort. Plans for action were formulated and attempts were made to request the funds needed through the two lead agencies, Agriculture and Commerce. (In effect, Agriculture has been the lead agency, with Commerce playing a secondary role.) A special linkage was formed with state agricultural research communities through a Guayule Administrative Management Committee (GAMC) comprised of representatives of the experiment stations and cooperative extension services of the states of Arizona, California, New Mexico, and Texas, plus a representative from the USDA, Agricultural Research Service. More recently a representative from the state of Mississippi joined the committee. The GAMC has worked closely with USDA program managers Howard Tankersley, Richard Wheaton, and Daniel Kugler.

Actual funds appropriated for guayule research and development, however, never reached a level anywhere near that envisaged in the original or reauthorized act. Funding for the program has been provided mainly by the USDA through a Special Grants Program of about $700 thousand per year administered by the Cooperative State Research Service (CSRS) and in-house research at about $1 million per year performed by the Agricultural Research Service (ARS). Total expenditures by the USDA on guayule from 1979 through 1988 have been about $17 million. To this figure must be added the following expenditures: NSF (1976-85)— $2.7 million, Department of Commerce—$0.90 million, Department of the Interior—$0.3 million, Department of Energy—$0.35 million, and Department of the Army—$0.3 million. Thus, the federal government has spent approximately $21.5 million since the inception of the new effort on guayule research and development. This does not include the administrative costs incurred by the participating agencies nor the costs of the substantial commercialization project initiated by the Department of Defense (estimated at about $25 million), which is treated separately in Chapter 15.

Investigators who have carried out basic and applied research on guayule ranging from genetics and biochemistry, plant breeding and agronomics, to rubber extraction and coproduct utilization have summarized their findings in chapters of this book. It is, therefore, a progress report on what we have learned about the science and the technology of *Parthenium argentatum*, especially in regard to its potential as a domestic source of natural rubber that will be economically competitive with the rubber tree, *Hevea brasiliensis*.

This is a good place for me to stop and for you to take over . . . .

H.T. Huang
National Science Foundation

Chapter 1

The History of Rubber

James Bonner

The history of rubber comprises a portion of the history of the New World (1). Rubber balls were used as playthings by the Indians of the Americas at least as far back as the Mayan civilization of Yucatan and Guatemala over a thousand years ago. The Aztecs played a kind of tennis with rubber balls at the time of the Spanish conquest of Mexico, which concluded in 1519. A team of Aztecs was brought to Spain to play tennis before the Spanish Emperor Charles V in 1524. But the Spanish were uninterested both in tennis and in rubber.

Two hundred years later, in 1736, the French scientist Charles Marie de la Condamine arrived in Quito, Ecuador, on an astronomical mission. He and a second French scientist, Pierre Bougerer, who was sent to a second position also on the equator and some distance from Quito, were simultaneously to measure the angle of a given star at a predetermined date to determine the arc of the meridian and hence the diameter of the world. This was successfully completed, although it was 15 years or so before the two scientists were reunited. The earth proved to be round.

From Quito, la Condamine descended the Amazon all the way to its mouth. On arriving in Amazonia he discovered that flasks for carrying water were made from rubber latex. This same latex was used to waterproof boots and to make waterproof tents and cloaks. La Condamine made some of these items for himself. As he descended the Amazon, la Condamine found candles made of rubber. The candles were made of long, cylindrical pieces of coagulated latex

which burned for a very long time and gave a useful light. Never, though, did he find the tree that produced the latex used by the Indians to make the waterproof articles. Ultimately it was a French engineer, Francoise Fresneau, who, with the help of Indians whose language he had learned, found the latex-producing tree in French Guiana, near Cayenne, and named it the *caoutchouc* ("weeping tree" in the Indian dialect). Small samples of rubber from the caoutch-ouc tree were brought to Europe intermittently by returning explorers. Joseph Priestly in England found that pieces of caout-chouc were useful to erase pencil marks and thus launched the first commercial use of rubber. In London rubber was sold for use in erasers starting at the end of the 1700s. In the Indian languages of Amazonia the tree yielding rubber latex was called *heve*. In 1755, therefore, the French botanist, Aublet, named the genus *Hevea* and at the Kew Botanical Garden and Herbarium in London the tree was named *Hevea brasiliensis*.

Uses of Rubber

The first large-scale use of rubber was developed by Charles McIntosh of Glasgow, who found that solid rubber could be dissolved in naphtha. He coated fabric with a solution of rubber in naphtha and then put two coated layers together so that the rubber races stuck to each other thus forming a sandwich from which he tailored waterproof cloaks. These were appropriately named "McIntoshes." McIntosh's business, started in Manchester in 1825, became a very large and profitable one.

At about the time that McIntosh was developing a large-scale use for rubber, Thomas Hancock in Britain discovered that fragments of rubber passed repeatedly through rotating rollers heated and coalesced into a homogeneous ball in which the original pieces could no longer be discerned. From this rubber ball lots could be cut to form sheets, erasers, or any desired form. Hancock set up a factory in which he used this machine, which he termed a masticator. With this masticator he could take random scraps of rubber in pieces of any shape as they were delivered from ships to England and form them into balls or cylinders which he

then cut into the desired, salable shapes. He attempted to combine his business with that of McIntosh, but McIntosh turned him down and Hancock started a competitive business, making waterproof clothing in France where McIntosh's patent did not pertain. McIntosh eventually gave in and the two firms merged.

In the United States, action in rubber also began early in the 1800s. Imports introduced rubber to the U.S. market. The first of these were rubber shoes made in Brazil. In 1826, 8 tons of the Brazilian rubber shoes were imported; by 1830 this number had increased to 160 tons. The United States began manufacturing it's own rubber shoes in 1834 in Roxbury, Massachusetts. Here also Charles Goodyear started experimenting on how to "cure" rubber to make it unresponsive to temperature changes. Goodyear, along with Nathaniel Hayward in England discovered that mixing sulfur with rubber, and then heating the sulfur-containing rubber caused the substance to retain its elasticity without stickiness. Hancock heard of this discovery from Goodyear and made a systematic study of how to incorporate sulfur into rubber. He discovered that sulfur could be integrated into rubber by masticating solid sulfur and rubber together, or preferably, by masticating the mixture at a temperature above the melting point of sulfur (113°C). Hancock found that rubber containing the incorporated sulfur became a solid when heated to a temperature above the melting point of sulfur. It retained its elasticity without stickiness and possessed the ability to preserve a preformed shape. Hancock dubbed this process vulcanization and obtained a patent for it in 1843. Although we have been led to believe that Goodyear invented vulcanization, it was in fact Hancock who made vulcanization a useful procedure.

The invention of vulcanization brought with it many new uses for rubber. Among these were the following: insulation for electrical wires (1840), solid rubber carriage tires (1845), and railway car bumpers (1845). By 1870 rubber was used in the United States and in Britain for most of the purposes we know of today, with one exception.

The largest use of all was still to be developed, to wit, the pneumatic tire. The bicycle, invented in France by Ernest Michaux in 1855, initially rode on solid rubber ties. Developed in

its present form by Rover in Britain in 1885, the bicycle was provided with pneumatic tires invented by John Dunlop in Britain in 1888. Bicycle tires became by far the largest market for rubber.

Just as the bicycle consummated its conquest of the world, the automobile was beginning its rise to popularity. The first automobile was made by Daimler-Benz in Germany in 1886. Edouard Michelin in France invented the pneumatic automobile tire in 1895. The first American pneumatic automobile tire was made by Benjamin Goodrich in 1896 for the Winton automobile, which is no longer with us. Harvey Firestone's Tire and Rubber Company (now Bridgestone/Firestone) was founded in 1900 and located in Akron, Ohio, which soon became the pneumatic tire capital of the United States.

Firestone had been a friend of Henry Ford for some time. Ford's Model T appeared on the U.S. market in 1907 and revolutionized our world. When Firestone heard of Ford's automobile company he boarded a train from Akron to Detroit, Michigan, for a reunion with Ford and returned with a contract for tires for the Model T Ford. The contract was for an initial 20,000 tires, but during 1908 approximately 135,000 tires were delivered to Ford and to other automobile manufacturers. Parenthetically, Frank Seiberling in 1898 founded a company in Akron to which he gave the name of Goodyear Tire and Rubber Company. Goodyear, already long gone, has as his memorial what is now the world's largest tire company.

The Growth of the Rubber Industry

By 1870 the amount of rubber exported from Amazonia had grown to 15,000 tons per year, a 20-fold increase from 750 tons per year in 1850. It had taken rubber production over 100 years to grow from near 0 at the time of la Condamine in 1736 to 750 tons a year in 1850. The increased growth rate during the succeeding 20 years was but a foretaste of what was to come.

By 1890 exports of rubber from Amazonia and all other sources rose to 29,000 tons; double the amount exported in 1870. By 1910, exports had tripled to 94,000 tons, and by 1930 they

had almost tentupled to 917,000 tons. The rapid growth in rubber production from Amazonia and other sources can be attributed principally to the automobile. Automobile numbers grew much more rapidly in the United States than anyone had expected and much more rapidly there than in any other region in the world. From 1910 until 1942, over half of all the rubber produced in the world came to the United States. All of this was natural rubber. Synthetic rubber became a factor only during and after World War II (2).

Collecting Rubber

In the early days collection of rubber was an exceedingly labor-intensive and poorly paid occupation. It was carried out in primitive conditions and in an exceedingly remote and inaccessible region. The rubber collector, a penniless peasant, was signed on by a rubber dealer who then turned him over to an intermediary who took the about-to-be-tapper up river, showed him a hut on the river where he should bring his collected rubber, gave him three months of food on credit, and sent him out into the jungle where he first built himself a cabin. Next he looked for and found a rubber tree which he slashed with a hatchet or machete. He then collected the latex, and moved on to the next tree along a path that he had previously made to connect the trees in his tapping area. With the hatchet he cut two grooves in the bark, each at an angle of 45° from the vertical and connected at the bottom. The latex flowed down the "V" and spilled off into a coconut husk or metal cup at the bottom of the "V." In this way the tapper collected an average of 20 g of rubber per tree per tapping and could collect as much as several hundred grams of rubber per day. Because of the distance between trees he spent a great deal of time walking. On the next day the tapper returned to his tapped trees, made fresh cuts 10 cm above the previous ones, and repeated the procedure.

Each evening the tapper in his forest hut pooled his latex in a large basin and dipped a wooden paddle into it so that the paddle became coated with latex. The paddle was then smoked over an open fire; the temperature of the smoke being about 65° C. The smoking

coagulated the latex and dried the rubber. On succeeding days more latex was similarly treated until a ball of rubber weighing up to several kilograms had accumulated on the paddle. This ball of rubber was then ready to take to the delivery station on the river. In this way natural rubber was collected and processed, first by the native Indians and subsequently by the thousands of tappers who together produced up to a maximum of 50,000 tons of rubber per year from Amazonas.

Much more efficient rubber collection came only later, after the development of an understanding of latex flow and particularly after the conversion of the rubber tree from a wild into a cultivated plant grown in plantations. As a result of these improvements, the rubber tapper spent much more time tapping and much less walking from tree to tree.

Rubber-Producing Plants

The rubber exported from Amazonas to England and other countries was all harvested from *Hevea brasiliensis* and other related species growing in the wild. The Amazonian tropical forest in which *Hevea* is found consists principally of slightly elevated uplands between rivers with good drainage and of a very high density and variety of tree species. On the average about four to eight *Hevea* trees are found per hectare.

Hevea is not the only plant that produces rubber—there are about 2,500 rubber-producing species. These species are scattered through a large number of unrelated families of higher plants and are believed to represent independent experiments by nature designed to find out whether the basic isoprenoid monomer used by all living things to make steroids, carotenes, and essential oils, among other things, can be used for something further. In many cases the rubber is formed as microscopic particles suspended in the cytoplasm of the cell together with other cellular components such as mitochondria, lysosomes, and enzymes, the whole forming a turbid, milky suspension or latex.

In many plants, as in *Hevea*, the bark cells containing the latex are interconnected so that when a cut is made latex flows not only from the cut but toward the cut through the network of

latex vessels. In the case of *Hevea brasiliensis* the latex flows from the cut for an hour or more, from as much as a meter away. The latex, however, ultimately coagulates, stopping the flow. *Hevea brasiliensis* distinguishes itself from all other lactiferous plants by producing fresh rubber to replace that lost by the tapping. Within 48 hours of a tapping cut the latex of the tapped bark is replenished and contains 35 percent by weight of rubber just as it did before tapping. *Hevea* may be tapped in this way every other day for 40 years or more without any major diminution in yield. It is this response to rubber harvest by new rubber synthesis that sets *Hevea* apart from all other rubber-producing plants. The vast majority form latex cells or vessels and fill them with rubber during the growth of the latex-containing cell. This cell once tapped, however, remains empty. Rubber can be, as it were, mined from most species; only *Hevea* can be milked.

The conclusions from these facts were 1) that it is very difficult to harvest rubber from wild forest *Hevea* trees and 2) that there are no obvious replacements for *Hevea* as a source of rubber. This led to the inevitable conclusion that *Hevea* must be grown as a cultivated crop.

Rubber Plantations

The industrial applications of rubber were discovered by McIntosh, Hancock, Dunlop, Michelin, Goodrich, Goodyear, et al., and as a result exports of rubber from Amazonia steadily increased reaching, as we have seen, 15,000 tons per year by 1870. Dunlop's invention of the pneumatic bicycle tire in 1889 not only greatly increased the appeal and the sales of bicycles, but also greatly increased the requirement for rubber imports into Europe and America. In response, heroic efforts in Amazonia resulted in ever-increasing shipments, peaking at 49,000 tons per year in 1912. At the same time massive efforts were made in tropical Africa and in tropical Asia to mine the rubber-containing trees of those continents. The year of greatest production of wild rubber from areas other than Amazonia was 1910 at 39,000 tons per year, although this latter production quickly declined to zero. Since consumption in 1910 in the

United States alone equaled the entire Amazonian production, clearly some other source of rubber was seriously needed. Luckily this second source was already in place.

In London Sir Clements Markham, President of the Royal Geographical Society, and responsible for moving the quinine tree (*Cinchona succirubra*) from Brazil to India, noted the growing trade in wild rubber from Amazonia and undertook to similarly move *Hevea brasiliensis* from Brazil to Asia. The first two trials initiated by Markham in 1872 were unsuccessful. The initial trial involved moving seeds to Kew Gardens where they were germinated and sent as plants to India. There they died from drought and low humidity. The second trial sent seeds to Calcutta where they failed to germinate. *Hevea* seeds remain germinative for only a few weeks and the seeds had been sent, unluckily, by sailing ship. They were nongerminative by the time the ship arrived in Calcutta. Markham then wrote in 1876 to Henry Wickham, explaining his desire to bring the plants to Asia and authorizing "wide discretion" in expenditures. Wickham was a most suitable person for the job. He was at the time living in Brazil. He was an adventurer and the author of a travel book on Brazil. He was also a plant collector who had from time to time sent seeds of interesting plants to Kew Gardens. Best of all he thoroughly understood the importance of Markham's request. He at once collected 70,000 seeds from *Hevea brasiliensis* trees. By good fortune he found an empty, large, new steel steam freighter, the "Amazonas," which was on its maiden voyage to Brazil. The captain had no return load and his ship was lying empty at anchor on the Amazon. Wickham chartered the entire vessel, filled it with his 70,000 seeds, and called on the governor of Para (a state of Brazil containing the city of Belem, which was the central collecting point for rubber and its shipment to markets in Europe and the New World). Wickham asked for the governor's help in expediting the departure of his ship for England as it carried "delicate botanical specimens for delivery to his Brittanic Majesty's own Royal Gardens at Kew." This assured that when the ship docked at Belem near the mouth of the Amazon it was quickly cleared and left the port. The vessel made straight for Liverpool and the 70,000 seeds were sent to Kew where they arrived on 14 June 1876. Of the 70,000 seeds 1,900 germinated and were grown into seedlings. They were packed

in special cases developed for the purpose at Kew Gardens (so-called Wardian cases) and sent, in care of a Kew gardener, to Ceylon. The bulk of the seedlings were planted there in the botanical garden at Heneratgoda, where they thrived. A few of the plants remain today. In 1976 Sri Lanka (formerly Ceylon) held a great centennial celebration in honor of the arrival of the rubber tree in Asia.

The new trees thrived in Ceylon and were planted there on a large scale. They fortunately had arrived just in time to replace coffee which had been the mainstay of the Ceylonese economy, but which was being decimated by a new variety of coffee blight fungus. Trees were also sent from Ceylon to India near Calcutta and to Burma. In Burma they thrived, particularly in Tenasserim near the border of Thailand.

Two of the 40 cases of seedlings dispatched from Kew were sent directly to Singapore and specifically to the Singapore Botanical Garden. These plants died, however, from lack of care. In 1877, 22 plants were sent from Ceylon to Singapore. Wickham, who became Sir Henry as a result of his work, had been of the opinion from the first that the Malay Peninsula would be the ideal place to grow *Hevea* and he proved to be correct in his judgment. However, interest in rubber in Malaya did not take hold for almost 20 years.

In 1888 Henry Ridley arrived in Singapore to become the Director of the Singapore Botanical Garden. Ridley had been in Brazil, and saw how well his rubber trees had grown—his 22 trees had increased to over 1,000 by 1888. He took every opportunity to promote the establishment of plantations of *Hevea*. He systematically studied the soil and site requirements of the tree, and also studied especially diligently how best to tap *Hevea*—how to reopen a tapping cut, the best tool to use, how to cause the least damage to the tree.

Finally, after eight years of advertising, Ridley got a serious nibble. A wealthy Malaccan Chinese, Tan Chay An, asked for plants to establish a rubber plantation in Malacca. In 1896 Ridley gave him, free-of-charge, the plants required to establish 43 acres. At this time the price of Brazilian coffee was falling rapidly due to total political and fiscal disruption in that country. Malayan coffee could no longer be sold in competition with the low price of Brazilian coffee which was the preferred brew in Europe.

The price of rubber, on the contrary was high and was going up steadily. Soon every planter in Malaya started switching to rubber. Ridley exhausted his supply of seeds and seedlings and had to beg for resupply from Ceylon. The Dunlop Rubber Company, famous for its bicycle tires, began their own plantation in 1900. The rubber planting boom was on. A similar stampede toward rubber occurred at the same time in Ceylon.

In 1877 the Director of the Ceylon Botanical Garden sent a gift of a few rubber plants to the Director of the Botanical Garden at Buitenzorg (now Bogor) in Java. Further exchange of seeds took place informally; in particular, 33 seeds from 9 of the original 22 Singapore plants reached Buitenzorg from Penang in 1883. This was later considered to be a highly traitorous act. These 33 seeds formed the basis of the Indonesian rubber planting industry. Indonesia, a Dutch colony at the time, had a large population of scientists and a huge plantation industry. They also had many skilled people who knew how to establish a new crop and did so.

The first rubber from Southeast Asia to reach Europe consisted of 4 tons from Java. It was sold in Amsterdam in 1898. This rose to approximately 800 tons in 1900. Malayan rubber did not reach the European market in any considerable amount until 1903. However, the course of the future was clear. In 1910 wild rubber still made up almost 90 percent of total rubber production. By 1913 Amazonian production reached a peak of 50,000 tons per year, while plantation rubber from Malaya, Java, and Ceylon totaled 53,000 tons. Total world production and use of rubber by 1920 had reached 354,000 tons and included only 10 percent wild rubber. By 1930, wild rubber made up only 3 percent of total world production (Table 1).

Today the world is producing about 4.8 million tons of natural rubber per year. Of this Malaysia produces about 33 percent, and Indonesia and Thailand together produce another 45 percent. Production is, however, growing rapidly in both of the latter countries. Ceylon, India, Viet Nam, China, Liberia, and Brazil together supply about 20 percent of the total natural rubber of commerce (3, 4).

The world, as a whole, uses rubber today at the rate of about 15 million tons per year. What cannot be supplied by natural rubber is provided by a variety of synthetic polymers that have elastomeric qualities. These include *cis*-polyisoprene, which in theory should be like natural

Table 1. World rubber production 1900-1990.

	Total produced and consumed ('000 tons)	Natural rubber produced and consumed ('000 tons)	Natural rubber market share (%)
1900	53	53	100
1910	102	102	100
1920	302	302	100
1930	722	722	100
1940	1,127	1,127	100
1950	3,339	1,750	75
1960	4,400	2,095	48
1970	8,625	2,990	35
1980	10,395	3,370	30.2
1990[a]	15,156	4,774	31.5

[a] Estimated.

Sources: Coates, 1987; Allen, Thomas, and Sekhar, 1973.

rubber, but is not, however, blessed with all the qualities of natural rubber. No synthetic polymer is yet capable of replacing natural rubber in a wide variety of applications, particularly those that require low heat buildup on flex. Natural rubber is needed for airplane tires, sidewalls of radial tires, large truck tires, and off-road, heavy-duty tires, for example. The proportion of natural rubber in the global elastomeric mix has remained steady at about 31.5 percent for the past dozen years and there is no present visible reason why this should change. The invention of synthetic elastomers produced a challenge to natural rubber. Natural rubber has, however, survived the challenge. In addition, since there is no evident way in which natural rubber production could have met the total elastomer demand of the automobile industry of the world, it is fortunate indeed that synthetic rubber appeared at just the needed time (Table 2).

Table 2. World distribution of natural rubber production 1990.

Region	Tons/y
Malaysia	$1{,}510 \times 10^3$
Indonesia	$1{,}180 \times 10^3$
Thailand	$1{,}040 \times 10^3$
All other	$1{,}040 \times 10^3$
World total	$4{,}770 \times 10^3$
World demand	ca. $5{,}000 \times 10^3$
World demand for synthetic rubber	ca. $10{,}000 \times 10^3$

Sources: Smit, 1982; Sekhar, 1983.

Guayule Rubber

Just as *Hevea* served as a source of rubber for Amazonas, guayule (*Parthenium argentatum* Gray) served as a source of rubber for the more northerly ball-playing Indians. It was general knowledge that the plant contained rubber. However, no commercial exploitation of guayule occurred until the rubber boom in Amazonia and the high price of imported rubber in the United States attracted attention to the supply at our doorstep.

During the period 1902-05, a number of methods for extraction of rubber from wild guayule were investigated (5, 6) including extraction by solvents (7, 8). The most successful method was devised by William Lawrence and adopted by the Continental Mexican Rubber Company. It involved the use a pebble mill to grind the guayule shrub and a water flotation method to remove the rubber worms that formed. In 1904 the first 50 pounds of guayule rubber were shipped from Mexico to the Manhattan Rubber Company in New York. It was found to be as satisfactory for use as *Hevea* rubber. A large extraction plant was then constructed in 1905 by

the Continental Mexican Rubber Company in Torreon, Mexico. That plant produced 8 tons of rubber per day (7). By 1907, Continental and other business interests accounted for a total of 20 extraction plants in Mexico including those in operation, shut down, or being built. Plants were built in many other locations in Mexico including Saltillo, Ocampo, Gomez Palacio, La Grunidora, Parras, Las Delicias, Cuatro Cienegas, Jimulco, and Cedral. In 1909, a plant was constructed in Marathon, Texas. Commercial production started in 1906 and from then through 1908 about 9,000 tons of rubber were shipped to the United States (9).

In 1909 eight factories of the Madero interests running night and day reached a total production of 350 tons of rubber per month (10). The bulk of the fuel used in these factories was the bagasse or refuse left after extraction. Large areas of native shrub were depleted, which was cause for concern. Unexploited shrub was limited and restoration by natural methods was extremely slow. In 1906, one estimate placed the total amount of shrub available at 489,000 tons (7) possibly equivalent to around 29,340 tons of rubber if the rubber content had been 6 percent. Guayule made an excellent smelter fuel and Guggenheim's, in their great mining smelters, burned 10,000 tons of shrub containing $500,000 worth of rubber. Guayule rubber production continued and rose to a maximum of 10,000 tons per year in 1910, but declined to 7,000 tons by 1912. The decline was not due to shrub depletion but was attributed to revolutionary disturbances in Mexico, which halted guayule rubber production. At the year of maximum guayule rubber production (1910), the guayule product made up 24 percent of the rubber imported into the United States.

The Continental Mexican Rubber Company, because of the disturbances in Mexico, moved its headquarters and plant to Salinas, California, and became the Intercontinental Rubber Company. In Salinas, methods for the cultivation of guayule were developed and desirable strains of guayule were selected. The Intercontinental Rubber Company planted 8,000 acres of guayule. During the late 1920s Intercontinental again started producing guayule rubber, this time from guayule plantations, at the rate of about 1,400 tons per year. Production ceased at the beginning of the Great Depression. It continued later on a very small scale until in 1942 the

company was taken over by the U.S. government when the Emergency Rubber Project (ERP) was initiated. During the four years of its existence the ERP produced another 1,400 tons of guayule rubber. It produced an additional 10,000 tons of rubber in guayule planted, grown to maturity, and harvestable; this was destroyed with the termination of the ERP in 1946 (Table 3).

From what we know today, all functions of rubber in tires and other manufactured objects could be as well performed by guayule rubber as by *Hevea* rubber. Whether guayule can produce commercial rubber at a competitive price is a principal issue and perhaps the only issue in determining the feasibility of developing a domestic natural rubber supply. The *Hevea* cultivars of today with today's agronomy produce an average of about 2,000 Kg/ha/y of rubber. Guayule, which does not yet have the history of selection and plant breeding that *Hevea* enjoys, is hard pressed to produce one-twentieth of this yield. The financial feasibility of guayule cultivation in the United States stands or falls on yield-per-acre-per-year.

In 1925 the Rubber Research Institute of Malaya (now of Malaysia) was formed with headquarters in Kuala Lumpur. The Institute rapidly grew into the largest research organization

Table 3. Estimated amount of guayule rubber delivered to the United States from Mexico 1900-1915.

Year	Tons	Year	Tons	Year	Tons
1900	0	1908	5,000	1912	7,000
1904	0.05	1909	7,000	1913	0
1906	1,000	1910	10,000	1914	0
1907	3,000	1911	9,000	1915	0

Sources: Lloyd, 1911; National Academy of Sciences, 1977.

in Asia and is still a premier example of an agricultural research organization devoted to the improvement of a single crop, *Hevea brasiliensis.* It is responsible for over 60 years of continuous improvement of rubber yield by scientific plant breeding and for the transformation of a product of variable and unreliable quality into a product of uniform, technically specific, and guaranteed properties. More than any other single organization or factor it is responsible for the continued viability of the natural rubber industry today. If guayule is ever to compete in the marketplace, it needs an analog of the Rubber Research Institute of Malaysia.

CONCLUSION

The story of rubber has been a long one, taking in as it has a period of almost 500 years. Discovery, a latent period of 200 years, rediscovery, invention of modest uses culminating in the invention of vulcanization 150 years ago, followed by the invention 100 years ago of useful devices that required vulcanized rubber. These devices are, of course, pneumatic tires and the wheeled vehicles that roll upon them. The first of these vehicles, and still an important factor, is the bicycle. The arrival of the second of these vehicles, the automobile, overwhelmingly influenced the development of the rubber industry. These two inventions have touched the lives of nearly every human being. The automobile has transformed our lives, our cities, and now our atmosphere. Once a luxury the automobile has now insinuated itself into the position of a necessity. Good or bad, we can't do without it. Our story of rubber illustrates how rubber, also first a novelty and then a luxury, has now become an integral component of our mobile, technology-based, human culture.

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Chapter 2

Biochemical Evolution and Species Relationships in the Genus *Parthenium* (Asteraceae)

Jan E. West, Eloy Rodriguez, and Ahmad Hashemi

INTRODUCTION

Guayule, *Parthenium argentatum* Gray, is a xerophytic shrub that is a dominant element of the flora on the limestone bajadas and hillsides of the Chihuahuan Desert of Northern Mexico and West Texas. Guayule is in the sunflower family of plants (Asteraceae), a large family with over 20,000 species. Plants of this family form a particularly conspicuous portion of the flora of the desert regions of southwestern North America (Figure 1). Several species of this family such as the Russian dandelion, *Taraxacum kok-saghyz*, and goldenrod, *Solidago* sp., have considerable amounts of rubber and have been investigated as potential commercial sources of rubber.

CLOSELY RELATED GENERA OF THE ASTERACEAE

The genus *Parthenium* is in the subtribe Ambrosiinae, the group that includes the ragweeds of the genus *Ambrosia*. The other genera of this subtribe are *Parthenice*, *Dicoria*, *Iva*, *Hymenoclea*, and *Xanthium* (1).

The genus *Parthenium* is unique in the subtribe in maintaining insect flower pollination. *Parthenium* pollen can be carried by the wind and some investigators have suggested wind pollination. However, flower heads do produce nectar and are commonly visited by insects, especially *Syrphid* flies (personal observation). The very closely related genus *Parthenice* (one species) does not produce nectar and has the pendulous capitulae characteristic of the wind-pollinated Asteraceae (Jan West, unpublished)). The two genera *Dicoria* (two species) and *Iva* (16 species) are wind pollinated and are similar to *Parthenium* in having capitulae with marginal seed producing florets and central staminate disk florets. The genus *Iva* includes species with characteristics that could be of economic importance to guayule in the future. Several species are halophytes; one species, *Iva frutescens* L., is a perennial that grows along the Atlantic coast from Nova Scotia to Texas. It is conceivable that new techniques such as protoplast fusion could transfer salt and/or cold tolerance from this species to guayule.

There are three other genera in the Ambrosiinae that are more distantly related to guayule. *Hymenoclea* (three species), *Ambrosia* (40 species), and *Xanthium* (two species) have the male flowers and the female flowers separated in distinct and separate heads. Most species of the genera are perennial shrubs of the desert of North America; other species include the ragweeds that cause respiratory allergies, and the common cocklebur of the genus *Xanthium*. The great majority of species of the Ambrosiinae have been studied chemically and found to accumulate large amounts of leaf sesquiterpene lactones similar to those found in the genus *Parthenium* (1).

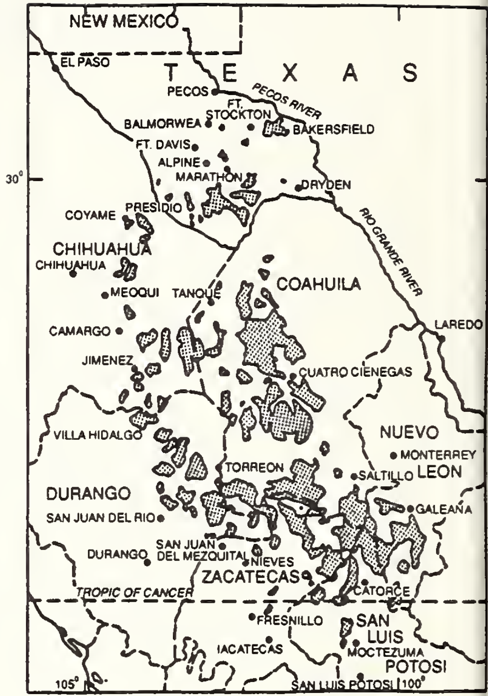


Figure 1. Distribution of native guayule in Mexico and Texas. (After Jenkins, 1946.)

THE EVOLUTION AND GENOMIC RELATIONSHIP IN PARTHENIUM

Evolutionary patterns and taxonomic relationships are important for the plant breeder interested in transferring specific traits to guayule. The chromosomes of more closely related species will pair more easily and there will be fewer problems of hybrid sterility. Chiasma frequency should also be greater in chromosomes that share more homology, thereby making it likely that novel

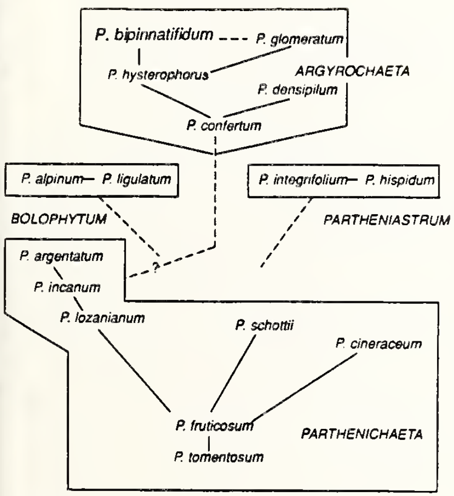


Figure 2. Hypothetical scheme of phylogenetic relationships in Parthenium.

recombinations with potential utility will occur more often. To state it more directly, it should be easier to transfer desirable traits from the species more closely related to guayule (2, 3).

Interspecific hybridization for the purpose of introducing desirable traits was a part of the Emergency Rubber Project (ERP) in the 1940s (4-7). Desirable traits of the treelike species investigated in the ERP include faster growth, greater biomass, and disease resistance. The plants of the genus *Parthenium* native to the northern United States have the ability to withstand colder winters, but were not investigated during the ERP. Other plants in the genus are herbaceous and grow more rapidly; thus, hybrids between them and guayule could grow faster than present guayule cultivars.

Rollins, in a 1950 monograph, discussed his observations of evolutionary trend in the genus. This monograph has a detailed description of the different species of the genus and includes a hypothetical scheme of the phylogenetic relationships in the genus (6) (see Figure 2).

Rollins based his theory of species relationships on the morphology of herbarium- and greenhouse-grown plants. Rollins hybridized *P. tomentosum* DC. var. *stramonium* (Greene) Rollins, *P. incanum* H.B.K., and *P. hysterophorus* L. with guayule. Many of these hybrids were backcrossed to guayule, however no analysis of chromosomal pairing of these hybrids was published. Rzedowski collected and described a new species of the genus which he named *P. rollinsianum* Rzedowski in honor of Rollins (8).

Healey and Mehta studied the leaf trichomes of the section *Parthenichaeta* (9, 10). All species of the section *Parthenichaeta* contain simple, uniseriate, cylindrical trichomes, and biseriate glandular trichomes. The small trees *P. tomentosum, P. schottii* Greenm. ex Millspaugh and Chase, and *P. fruticosum* Less. have the greatest variety of trichomes found in the genus. This variety includes one type, the prominent uniseriate conical trichome, that is unique to these three species. Guayule is covered with a layer of T-shaped trichomes, a type not found in other species of the genus. *Parthenium incanum* and *P. rollinsianum* have a leaf surface covered with very long uniseriate, whiplike trichomes, a type found at lower frequency in the arborescent species. Trichome morphology therefore indicates that the desert species are more

specialized and are the evolutionary descendants of the primitive treelike species.

Waines, Youngner, and associates at the University of California at Riverside have hybridized several species of the genus *Parthenium* with guayule and have studied the chromosomal pairing and fertility of these hybrids. Most investigators would agree that chromosomal pairing and fertility of interspecific hybrids are the best criteria for establishing species relationships (2, 3). Chromosomal pairing and fertility with guayule are also important criteria in establishing the utility of species as breeding stock.

Table 1. Frequency of unpaired chromosomes (univalents) in the F_1 hybrids between diploid *Parthenium argentatum* (2n = 36) and diploid species of the genus *Parthenium*.

Hybrids	Range	Mean
P. argentatum x *P. hispidum* var. *auriculatum*	0-9	4.43
P. argentatum x *P. tomentosum* var. *stramonium*	1-8	4.36
P. argentatum x *P. schottii*	0-8	3.92
P. argentatum x *P. fruticosum* var. *fruticosum*	0-7	3.21
P. argentatum x *P. alpinum* var. *alpinum*	0-6	2.46
P. argentatum x *P. ligulatum*	0-5	2.10
P. argentatum x *P. rollinsianum*	0-5	1.54

Parthenium rollinsianum appears to be the species most closely related to guayule (11). The two caespitose species *P. alpinum* (Nutt.) T. & G. and *P. ligulatum* (Jones) Barneby also appear to be close relatives of guayule (11, 15). *Parthenium fruticosum* and *P. schottii* apparently are more closely related to guayule than *P. tomentosum*; this is in agreement with Rollins (12, 13). Section *Partheniastrum* is more distantly related to guayule, but still retains sufficient homology to make it possible to hybridize diploid *P. hispidum* Raf. var. *auriculatum* (Britt.) Rollins and diploid guayule (14). Despite a low level of fertility it has been possible to make backcrosses from this hybrid to guayule at the diploid level.

It has also been possible to make hybrids between sexual, artificially induced tetraploid guayule and two natural tetraploid species of the genus *Parthenium, P. integrifolium* L., and *P. confertum* Gray. These F1 hybrids behave as amphidiploids with an average of 33.61 and 33.44 bivalents at diakenesis respectively (16). This lack of multivalent association in the hybrids may be interpreted as a lack of homology between the genomes; this is in agreement with their markedly different morphology. It is also possible that the preferential pairing of the parental chromosomes has prevented homologous pairing in the hybrids.

The exact position of *P. incanum* is still open to debate. No diploid populations of the species have been found, and all existing populations appear to be apomictic. Although Rollins made experimental hybrids of *P. incanum* with guayule, the polyploid and apomictic nature of hybrids lessens the utility of a cytogenetic analysis.

Rollins and others have assumed that *P. incanum* is the closest relative of guayule. However, natural populations of diploid guayule appear to maintain genetic purity when growing sympatrically with *P. incanum*; the introgressed populations of guayule are more common in the polyploid apomictic populations (4, 6, and Jan West, unpublished). These introgressed populations are likely to be the result of a hybridization in the distant past that has been maintained by several generations of apomictic reproduction. Naqvi experienced difficulty in making greenhouse crosses between diploid guayule and *P. incanum* (personal communication). Rodriguez and West noted the chemical and morphological similarities between *P. incanum* and the herbaceous species of section *Argyrochaeta* (1, 17).

Hybridization between guayule and the herbaceous species of section *Argyrochaeta* has only been possible at higher levels of ploidy (4, 16). This difficulty and the differences in morphology, basic chromosome numbers, and chemistry suggest that this section is only distantly related to guayule. However, hybridization is possible and it should be possible (but difficult) to transfer genes from these plants to guayule.

Two species of the genus *Parthenium, P. cineraceum* Rollins and *P. glomeratum* Rollins, are endemic to South America and have not been accessible to guayule researchers. Two other

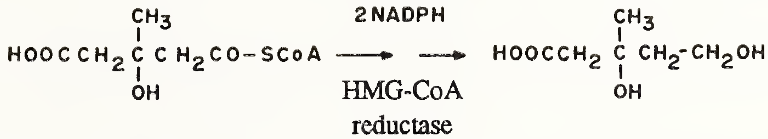
species, *P. lozanianum* Bartlett and *P. densipilum* Blake, are extremely rare and have not been collected or studied recently.

BIOCHEMICAL SYSTEMATICS AND TERPENOIDS IN PARTHENIUM

Modern systematics uses all the available information about plants to determine species relationships. The distribution of plant secondary metabolites has provided information that is useful in determining species relationships in the genus. Knowledge about the types of secondary metabolites in *Parthenium* is crucial to all guayule investigators since rubber and resin are the secondary metabolites of guayule. Insect feeding has also been correlated with specific chemicals in *Parthenium* (18).

Guayule is most famous for accumulating large quantities of polyisoprene polymers (i.e., rubber). Guayule and other members of the genus *Parthenium* produce large quantities of other compounds of isoprenoid origin as well. Approximately 40 percent of the resins that are coproducts of guayule processing are chemicals of isoprenoid origin. The volatile constituents that give a guayule field its distinctive odor are terpenoids of isoprenoid origin. Some studies have indicated that an acre of guayule can produce 1,000 lb of these volatile constituents in one year. Other isoprenoids in guayule will undoubtedly be shown to be factors in resistance to insects and disease. One isoprenoid compound from guayule has been shown to cause allergenic contact dermatitis (19).

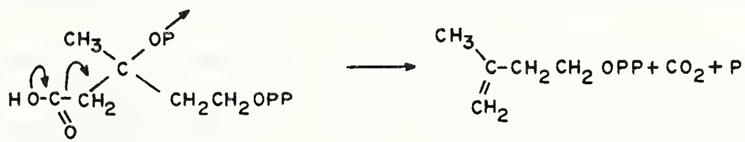
Isoprenoid compounds (or terpenes) are the products of a series of reactions that have mevalonic acid as an intermediate. The growth regulator that stimulates rubber accumulation, 2-(3,4-dichlorophenoxy)ethyldiethylamine (DCPTA) was initially postulated to stimulate the enzymes of this pathway (20). Lynen showed that this was the rate-limiting enzyme in terpenoid and rubber production in *Hevea* was ß-hydroxy, ß-methyl glutaryl CoA reductase (21).



ß-hydroxy, ß-methyl glutaryl CoA

mevalonic acid

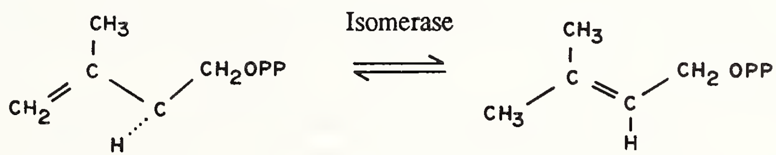
Phosphorylation of mevalonic acid and a concerted decarboxylation-dehydration reaction of the activated intermediate produce isopentenyl pyrophosphate (IPP).



mevalonic acid 5-pyrophosphate

isopentenyl pyrophosphate

IPP is the biogenetic precursor to isoprene compounds. Isopentenyl pyrophosphate can be converted to dimethylallyl pyrophosphate in an isomerization reaction that requires no energy input.



isopentenyl pyrophosphate

dimethylallyl pyrophosphate

Dimethylallyl pyrophosphate is an ideal electrophile, and isopentenyl pyrophosphate is an excellent nucleophile; the product of the reaction is geranyl pyrophosphate, the precursor of monoterpenes. Monoterpenes are produced in large quantity by guayule and other species in the genus. The small size of the monoterpene molecules and their nonpolar nature makes these compounds volatile; that is, they are gases at room temperature. Monoterpenes are the main constituents of the odors we associate with flowers or leaves and as such are commonly called essential oils. Many spices (basil, sage, rosemary, thyme, etc.) have large amounts of monoterpenes; other monoterpenes are constituents of perfumes harvested from flowers. Monoterpenes have biological properties and can function as repellents to plant-eating animals such as insects, sheep, goats, and cattle. Kumamoto, Scora, and Clerx have investigated the monoterpenes of *Parthenium* (22).

Extension of the geranyl unit by the addition of an isopentenyl unit produces the C_{15} compound farnesyl pyrophosphate, the progenitor of sesquiterpenes. Sesquiterpenes are produced in copious amounts by all species of *Parthenium.* The simple unoxygenated sesquiterpenes is volatile with properties similar to monoterpenes. The oxygenated and more modified sesquiterpene lactones are the principal terpenoid constituents of the leaves of all species of *Parthenium*, except guayule and *P. rollinsianum* (1). Many sesquiterpene lactones function to repel herbivores and one sesquiterpene lactone compound, parthenin, has made *P. hysterophorus* a notorious cause of allergenic contact dermatitis (23). Interspecific hybrids of guayule with other species of *Parthenium* also contain sesquiterpene lactones; these compounds could be factors conferring insect- or disease-resistance to future guayule cultivars developed from these hybrids (18). These sesquiterpene lactones could also cause problems of contact dermatitis or lung toxicity for farm workers (23).

Rodriguez investigated the sesquiterpene lactones and flavonoids as systematic markers in the genus *Parthenium* (1). Rodriguez concurred with Rollins' view that the small woody trees of section *Parthenichaeta* were the most "primitive" members of the genus. *Parthenium tomentosum* and *P. fruticosum* were both found to contain the three major types of sesquiterpene

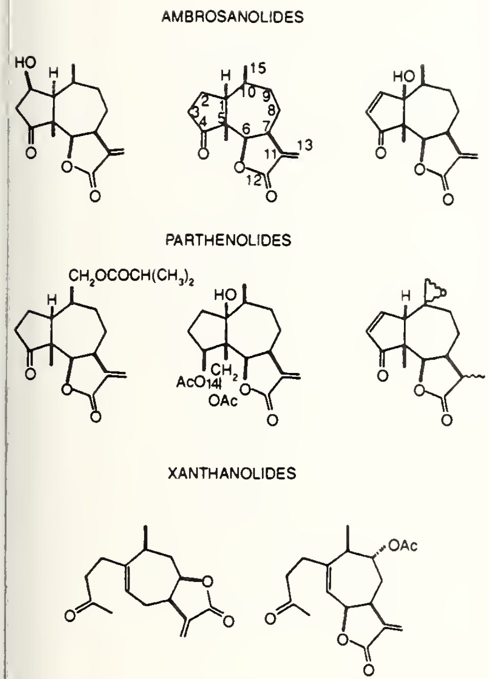
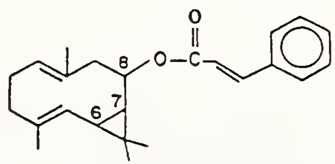


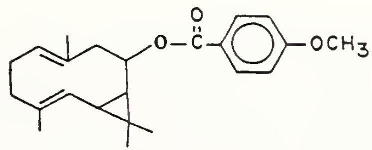
Figure 3. Sesquiterpene lactones from Parthenium.

lactones; ambrosanolides, xanthanolides, and parthenolides. *Parthenium incanum* and the herbaceous species of section *Argyrochaeta* were found to contain mostly ambrosanolides. The species of the northern sections *Bolophytum* and *Partheniastrum* contain only parthenolides. *Parthenium rollinsianum* and guayule were the only species to completely lack sesquiterpene lactones. This pattern is consistent with Rollins theory that the treelike species are "primitive" and all other species are specialized derivatives (Figure 3).

Guayule does contain large quantities of a sesquiterpene cinnamic acid ester, guayulin A. One study with guinea pigs found that the guayulins can cause a severe contact dermatitis (19). Fortunately subsequent studies with human subjects indicate that the guayulins are very weak sensitizers. Guayulins are a major constituent of guayule resin (10-15 percent) and any commercial use for them would enhance the prospects of commercializing guayule.



Guayulin A

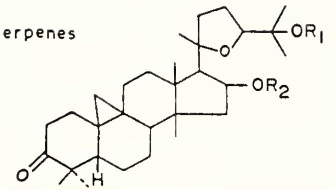


Guayulin B

Extension of the farnesyl pyrophosphate by addition of another isopentenyl unit yields the C_{20} compound, geranylgeranyl pyrophosphate; the progenitor of diterpenes. These compounds have not been reported to be common constituents in the genus *Parthenium*. The plant growth hormone gibberellic acid is a diterpene.

Two farnesyl pyrophosphates dimerize tail to tail (the CH_2OPP end is the tail, and the two methyl groups form the head) to form a triterpene. Triterpenes are ubiquitous as steroids; for example, cholesterol, vitamin D, testosterone. Triterpenes such as argentatin A are the most abundant terpenoids in guayule resin, (20-30 percent), and any market that could be developed for them would enhance the possibility of making guayule a commercial crop (24).

Triterpenes



Argentatin A R_1=R_2=H
Argentatin B R_1=A_c:R_2=H
Argentatin C R_1=R_2=A_c

Two molecules of geranylgeranyl pyrophospate dimerize tail to tail to form a tetraterpene; the precursor to carotenoids. Carotenoids are accessory pigments of photosynthesis and are also important in reducing free radicals. Vitamin A has a tetraterpene precursor. DCPTA was originally synthesized to stimulate production of carotenoid pigments in citrus fruits (20).

RUBBER AND POLYPRENOLS

Higher terpenes or polyprenols are less common and have not been well characterized. Polyprenols are composed of varying numbers of isoprene units; for example, ficaprenol, the common polyprenol found in fig trees, can contain 10, 11, 12, or 13 isoprene units. One polyprenol, dolichol (which contains 20 isoprene units), is a cofactor necessary for the biosynthesis of glycoproteins; the antigen receptors necessary for cell recognition and antibody binding are glycoproteins.

Tanaka studied the stereochemistry of polyprenols by carbon-13 nuclear magnetic resonance spectroscopy. He found that all polyprenols have a terminal dimethyl allyl unit followed by three isoprenes with a head to tail *trans* stereochemistry (25). All isoprene units added after this have a *cis* stereochemistry. Geranylgeranyl pyrophosphate (which has an all *trans* stereochemistry) is thus the precursor to polyprenols. These compounds are then elongated by *cis*-prenyl transferase which catalyzes the stepwise addition of IPP molecules to the growing chain.

Tanaka also studied the stereochemistry of rubber polymers from guayule and *Hevea*, and he found them to be identical with polyprenols; one dimethylallyl, 3 *trans*, and many *cis* isoprene units (26). This fact makes it attractive to postulate that *cis*-prenyl transferase and rubber transferase are very similar or perhaps even identical enzymes. Both enzymes have the same primer, geranylgeranyl pyrophosphate, and both enzymes catalyze the same reaction, stepwise stereospecific addition of IPP. The only difference is the greater length of the rubber molecule, and this could be due to some simple mutations in the portion of the enzyme that controls chain termination. This conjecture is supported by the Gaussian distribution of molecular weight of rubber polymers. In contrast proteins and essential metabolites are polymers with clearly determined lengths. Rubber is probably not metabolically important, and chain termination is, therefore, a stochastic process determined by stearic interactions between the rubber molecule and a modified *cis*-prenyl transferase enzyme.

Over 2,500 species of plants and some species of fungi have been reported to contain rubber. It is our opinion that many more plants contain small quantities of rubber. Systematic investigations at the University of California, Irvine, have detected small quantities of rubber (mostly low-molecular-weight) in all species of *Parthenium* examined to date (27). Unfortunately, no investigation has been able to experimentally determine the physiological role for these polymers in higher plants. This strengthens the hypothesis that rubber might be the product of another enzyme such as *cis*-prenyl transferase and that it is not the product of a new enzyme, rubber transferase, that has appeared *de novo* in guayule and *Hevea*.

CONCLUSIONS

Recent research has provided new insights into the evolutionary patterns in the genus *Parthenium*. The interspecific hybridization program has given us a better idea of species relationships in the genus. Species relationships are important to plant taxonomists and plant breeders. Chromosomes of more closely related species have a larger number of chiasma and this

increases the probability of finding novel recombinations with better agronomic characterizations.

Investigations of the chemistry have elucidated the chemical structures of the compounds present in the resin of guayule and other species in the genus. This knowledge of the chemistry is essential if new and better techniques of separating resin and rubber are to be developed. A better understanding of natural product chemistry is also essential to any effort to develop a market for guayule coproducts. Natural chemicals present in the resin of *Parthenium* also can be dangerous to workers; or these same chemicals can deter noxious insects.

New insights into the inheritance of rubber quality and the biochemistry of rubber synthesis have also become evident from basic research. This information will be crucial to the effort to increase the yield of good quality rubber in guayule.

ACKNOWLEDGMENTS

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Chapter 3

The Morphology, Anatomy, and Ultrastructure of Guayule

Rachel Goss

MORPHOLOGY

Recent study of morphological features of guayule has dealt with trichome shape as an indicator of high or low rubber content (1). The results from two key studies will be the focus of the Morphology section (1, 2). Facts and theories originating in the work of these projects provide an important base from which to build a more complete understanding of the guayule plant. Over 75 native *Parthenium argentatum* Gray plants were examined and categorized into groups I, II, and III according to characteristic leaf shape, trichome morphology, and rubber content. Evidence indicates that varying degrees of introgression by *Parthenium incanum* H. B. K. (mariola), a plant that grows in close association with guayule in its native habitat, may be responsible for many of the morphological and biochemical differences among native populations (1).

Plants in group I are generally the smallest of the three groups and have the highest percentage of rubber, averaging 17 percent. Leaves of group I plants are oblanceolate, and leaf margins are entire to two toothed. Leaf length ranges from 2.5 to 4.3 cm and width from 0.4 to 1.0 cm. Both upper and lower epidermal leaf surfaces are covered with two types of trichomes. The most common type of trichome is nonglandular with peripheral cytoplasm and large central vacuoles. The stalk in group I trichomes is attached centrally to the cap cell. Generally cap cell lengths of T-trichomes of the upper epidermis are smaller than those of the lower epidermis, and trichomes

on the mid-vein or major veins have smaller cap cells. Most of the cap cells are blunt at both ends. Also present on both surfaces, interspersed among the T-trichomes, are four- to eight-celled glandular uniseriate trichomes.

Inflorescences in group I guayule are compound with a group of heads elevated on a leafless inflorescence axis ranging in length from 9 to 22 cm. Usually there is only one branch, near the tip, and the branch extends beyond the main inflorescence axis. The peduncles are from 1 to 2 mm and heads are compactly grouped.

Rubber content in group II plants is intermediary between groups I and III, with an average of 10 percent. Group II plants have narrow elliptic leaves, and the leaf margins are entire to four toothed. Leaf length ranges from 1.4 to 5.7 cm and width from 0.4 to 1.4 cm, therefore reaching greater length than group I leaves. The most common type of leaf trichome, present on both surfaces, is the T-shaped trichome; however, unlike T-trichomes of group I, the stalk is acentrally attached, and only one end of the cap cell is blunt, while the other end is straight or slightly hooked and pointed. Cap cell lengths of the upper epidermal T-trichomes are smaller than those of the lower epidermal surfaces. As in group I, some uniseriate four- to eight-celled trichomes are present in group II.

The inflorescence axis resembles those from group I, ranging from 9 to 20 cm in length, and branches once near the tip, with the branch extending beyond the main axis. The peduncles are short and the heads are compactly grouped.

Group III guayule plants contain the lowest amount of rubber in any of the groups, averaging 6 percent. Group III guayule plants also are more different morphologically than plants from groups I and II. Leaves from group II plants are ovate, and leaf margins are four to eight toothed. As in the groups previously described, T-shaped trichomes and uniseriate trichomes are present on both leaf surfaces. Stalks of T-trichomes are longer than those from the previous groups, one to three celled, and acentrally attached to the cap cell. The long end of the cap cell is pointed and wavy and the short end is blunt.

The inflorescence axis of group III is shorter than groups I and II (5-15 cm) and usually branches three or four times. All the branches are approximately the same height as the main

axis, and the peduncles are longer than the previous groups, making the cluster less compact.

Parthenium incanum (mariola) leaves are much shorter than leaves from groups I, II, or III, with leaf lengths ranging from 1.0 to 3.5 cm and widths from 0.7 to 1.8 cm. Mariola leaves are narrow elliptic to narrow obovate, with 4- to 10-lobed margins, the larger lobes sometimes bearing smaller lobes. Very long, uniseriate, and whiplike trichomes cover both leaf surfaces, and are interspersed with four- to eight-celled short, uniseriate trichomes like those found in guayule groups I, II, and III. A third type of trichome also present on the lower epidermal surface is multicellular and nonglandular at maturity.

The inflorescence is shorter than any of the guayule groups: 5-12 cm. The stem gradually merges into an inflorescence axis that branches more frequently than in guayule. Most branches are about the same height as the main axis, the peduncles are long, and the heads are loosely grouped. Mariola plants contain only traceable amounts of rubber, 0.3-0.5 percent, if at all.

Biochemical studies (1), which were conducted to detect sesquiterpenes in the three groups and in mariola, showed coronopilin, a sesquiterpene lactone, to be present in mariola but completely absent in group I guayule. Coronopilin was present at lower concentrations in groups II and III plants. It is suggested that groups II and III guayule plants are the result of introgression between guayule and mariola. Group II guayule plants composed over 50 percent of the samples taken and seem to be very well established throughout the area in Mexico in which guayule is distributed. The greater frequency with which group II guayule occurs suggests that group II guayule may have a high selective advantage. Group II and group III guayule, due to the amount of backcrossing with mariola, have a greater diversity of genetic makeup than does group I guayule (1). In native populations trichome morphology, leaf shape, and inflorescence morphology seem to be characteristics indicative of plant groups with similar genetic makeup, the groups probably the result of varying degrees of introgression with *Parthenium incanum*. Trichome morphology in particular seems to be a fairly reliable indicator of rubber content.

Selective crossbreeding of guayule, the only *Parthenium* species that produces more than trace amounts of rubber, with other *Parthenium* species has been pursued in an attempt to improve agronomic characteristics of guayule (2). The morphology of leaf trichomes of *P. tomentosum* DC., *P. fruticosum* Less., *P. schottii* Greenm. ex Millspaugh & Chase, and *P. rollinsianum* Rzedowski has been studied to help establish the relationship between trichomes of interspecific hybrids and their rubber content (2).

Four types of trichomes were observed in the species studied: nonglandular conical trichomes, nonglandular whiplike trichomes, glandular whiplike trichomes, and nonglandular cylindrical trichomes. The four species may be identified by trichome morphology, since the trichome complement for each is unique. All four types of trichomes are present on *P. tomentosum*, *P. fruticosum*, and *P. schottii*. Morphological differences within the trichome types, however, make it possible to distinguish one species from another. Biseriate glandular trichomes, though, were morphologically uniform and were noted in all four species, appearing more often on the lower epidermal leaf surface. Cylindrical, uniseriate trichomes were on both surfaces of *P. tomentosum*, *P. fruticosum*, *P. schottii*, and at very low concentrations on *P. rollinsianum*. Trichomes similar to the cylindrical, uniseriate type were also noted on *P. argentatum* and *P. incanum* (1).

ANATOMY

As discussed previously, guayule and its natural hybrids with mariola may be categorized into three major groups on the basis of leaf shape and trichome morphology (1). The stem anatomy of these three groups also has been found to differ significantly (3), as was determined in the study of stem samples that provides the basis of the Anatomy section. Stem samples were taken from erect branches over 1 cm in diameter. All ray cells from group I guayule were parenchymatous. Group II guayule plants occasionally developed secondary walls in the vascular

rays, and many ray cells of group III guayule were thick walled. The thick-walled cells are not capable of storing rubber, which is reflected in the rubber content of the three groups: group I guayule ranges from 14.8 to 19.5 percent, group II from 6.2 to 15.2 percent, and group III from 2.2 to 9.9 percent. Axial xylem parenchyma cells of group I plants occurred as two-cell strands with few fusiform axial parenchyma. Group II guayule plants have both two-cell strands and fusiform axial xylem parenchyma, and group III guayule have predominantly fusiform axial parenchyma with some two-cell strands. The pith in group I and group II guayule plants is entirely parenchymatous, while in group III guayule thick secondary walls were occasionally found in the pith. The pith diameter of group I plants was larger than that of group III, and the pith diameter of mariola was smaller than either groups I or III.

The average ratio of wood to bark in group I guayule is higher than either group II or group III (1:0.56 $\pm$ 0.14 vs. 1:0.43 $\pm$ 0.06 vs. 1:0.44 $\pm$ 0.06). Vascular ray height also varies significantly (as determined by the student t test) among the three groups and mariola: 677 $\pm$ 332 μm for group I, 580 $\pm$ 261 μm for group II, 533 $\pm$ 231 μm for group III, and 424 $\pm$ 234 μm for mariola.

Stem anatomical differences among the groups seem to be a result of introgression between mariola and guayule, as was noted in the trichome and leaf morphologies. Group III guayule is anatomically and morphologically more similar to mariola than groups I and II. The main characteristics group III and mariola have in common are lignified pith and vascular ray cells, fusiform xylem parenchyma, relatively fewer resin canals in the pith, and relatively shorter vascular rays. Most anatomical characteristics of group II guayule are intermediate between group I and III guayule plants, such as average vessel member lengths and diameters, vascular ray heights, average diameter of pith region, and the number of resin canals in the pith. The decreased average rubber content noted in guayule plants that have a higher degree of introgression by mariola may be the result of lowered rubber-storing potential exhibited by shorter vascular rays with schlerenchymatous cells and by a reduced xylem-to-phloem ratio (3).

ULTRASTRUCTURE

Ultrastructural study of stem parenchyma tissue, the site of rubber formation and storage (4, 5), provides insight into the mechanism of guayule rubber deposition. It has been shown that rubber formation in guayule is cyclic, with rubber formation stimulated by low, nonfreezing temperatures (6). Several studies have examined ultrastructural changes as a function of low-temperature stimulation in order to understand rubber deposition as it occurs in guayule (7, 8). Meristematic and basal stem sections of cortical parenchyma tissue from field-grown plants were examined before and after exposure to low, nonfreezing temperatures in order to understand rubber deposition and its relationship to the plant growth cycle (8).

Meristematic (young tissue on stem tips) cortical parenchyma cells from plants harvested in September, before the onset of low-temperature stimulation, synthesized very little rubber. The cellular constituents necessary for plant growth and for the production of rubber precursors are present in the parenchyma cells: nuclei, chloroplasts, frequently with starch grains as storage products, mitochondria, endoplasmic reticulum, and dictyosomes (Figure 1). Micrographs from meristems of non-cold-stimulated plants show only an occasional rubber particle (Figure 1). Nonstimulated meristematic cells contain two distinctive types of vacuoles: multivacuolate and univacuolate (Figure 2). Many vacuoles are contained in the cell in the multivacuolate condition, and cytoplasm is compressed at the vacuolar junctions (Figure 3).

The univacuolar cells contain one central vacuole surrounded by a peripheral band of cytoplasm (Figure 4). The fibrillar material in the vacuole may be the result of phagocytosis by lytic enzymes present in the vacuole. Multivacuolar and univacuolar cells occasionally appear side by side in the same stem cross section (Figure 2). Immature parenchyma of nonrubber-producing plants is composed of many vacuoles formed from cytoplasmic components that fuse to form one large central vacuole upon maturity (9). The existence of pockets of cytoplasm between vacuoles in guayule meristematic tissue (Figures 2, 3) suggests that the cells originally were composed of cytoplasm that was compressed upon vacuole formation. The emergence of

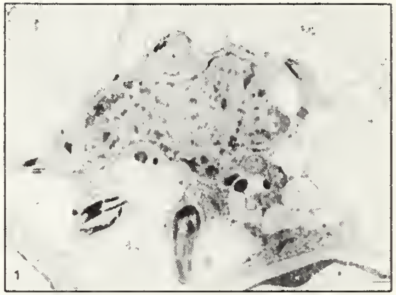

Fig. 1

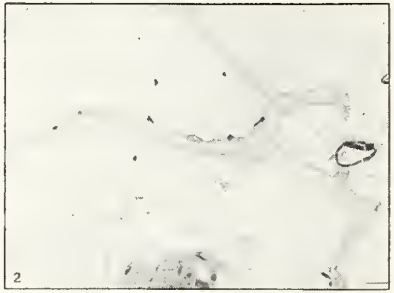

Fig. 2

Figures 1-2. 1) Cellular constituents such as a nucleus, peroxisome, chloroplasts, mitochondria, and dictyosomes are in meristematic cortical parenchyma tissue harvested prior to cold temperature stimulation. Bar represents 1.5µm.
2) Cells from non-cold-stimulated meristematic cortical parenchyma tissue are characterized by multivacuolar and univacuolar cells. Bar represents 3.0 µm.

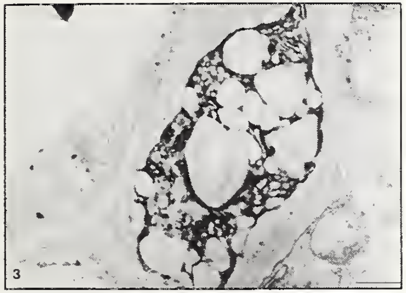

Fig. 3

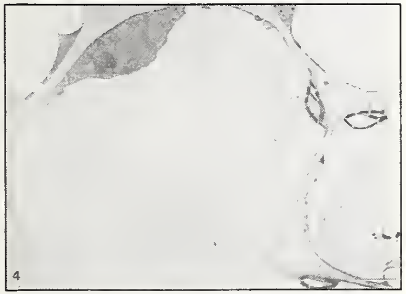

Fig. 4

Figures 3-4. 3) Varied sizes of vacuoles with cytoplasm compressed between them distinguish this cell from the meristem of non-cold-stimulated parenchyma tissue. Bar represents 0.8 µm. 4) One large central vacuole with fibrillar material in the interior and a thin layer of cytoplasm containing cellular organelles are the primary components of this cell from non-cold-stimulated meristematic parenchyma tissue. Bar represents 3.5 µm.

multivacuoles from cytoplasm in the meristem, and the presence of large central vacuoles, indicates a similarity in the development of guayule parenchyma with the parenchyma of nonrubber-producing plants—that is, plants not classified as *Parthenium argentatum* or any of its hybrids.

One large central vacuole characterizes the more mature basal tissue harvested before cold stimulation (Figure 5). The presence of large central vacuoles in older guayule stem tissue is a sign of mature tissue and suggests another developmental similarity between guayule and nonrubber-producing plants. The multivacuolar and univacuolar cells noted in the same cross section of meristematic tissue (Figure 2) may be indicative of tissue in the process of maturation. Most cells in the basal stem sections have one vacuole, and some of the cells have vesicles in the central vacuole (Figures 5, 6). Some of the vesicles appear to form by pinching off from the tonoplast (Figure 6).

A few rubber particles closely associated with the parietal cytoplasm are in the base of non-cold-stimulated plants (Figures 5, 6). The increased number of rubber particles in the base suggests that the mature tissue may be able to utilize metabolites for rubber biosynthesis that would be needed for growth in the meristem.

Plasmodesmata connecting cells are also apparent in Figure 6, a micrograph from nonstimulated tissue. Plasmodesmata occur frequently in both non-cold-stimulated and cold-stimulated tissue (10, 11) and would provide a mechanism for precursors to be shuttled from cell to cell. Numerous plasmodesmatal connections in guayule resin canals, phloem, and parenchyma tissue have been noted (11); it has been suggested that some rubber precursors pass from the resin canals to surrounding parenchyma and that metabolites processed in the resin canals originate in the phloem.

Cold-stimulated meristematic cortical parenchyma cells harvested in January exhibit a dramatic increase in rubber particle number over the nonstimulated meristematic cells (Figure 7). Rubber particles are in the cytoplasm alongside cellular organelles. Cells become increasingly full as rubber formation increases, and a dense osmiophilic substance is sometimes

Fig. 5

Figure 5. A few rubber particles are evident in the parietal cytoplasm in this cell from non-cold-stimulated basal cortical parenchyma tissue. Bar represents 3.5µm.

Fig. 6

Figure 6. Rubber particles of various sizes are located adjacent to cytoplasmic components in this cell from the basal cortical parenchyma of non-cold-stimulated tissue. Bar represents 1.4 µm.

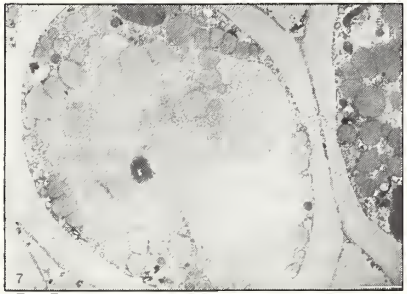

Fig. 7

Figure 7. Increased numbers of rubber particles in the cytoplasm distinguish cold-stimulated meristematic cortical parenchyma tissue. Bar represents 3.0 µm.

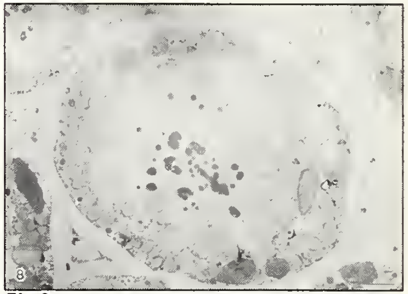

Fig. 8

Figure 8. Rubber particles of various sizes alongside cellular organelles are interspersed with a dense, osmiophilic substance in this cell from cold-stimulated meristematic cortical parenchyma tissue. Bar represents 4.7 µm.

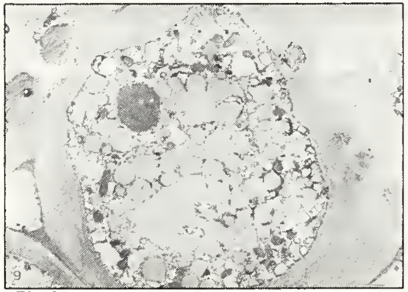

Fig. 9

Figure 9. Rubber particle proliferation and fusion of some particles characterize this cold-stimulated meristematic cortical parenchyma cell. Bar represents 4.64 µm.

Fig. 10

Figure 10. Increased rubber production is characterized by the rupture of vacuolar membranes and the extrusion of rubber particles into the vacuolar area. The tissue is from cold-stimulated meristematic cortical parenchyma. Bar represents 2.0 µm.

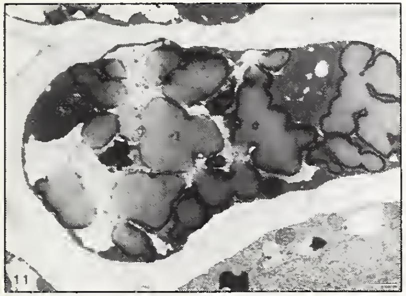

Fig. 11

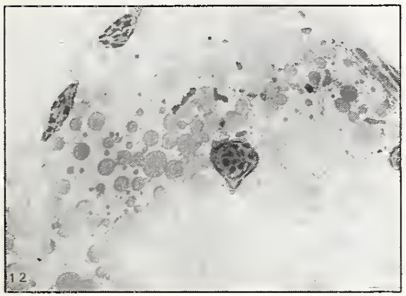

Fig. 12

Figures 11-12. 11) Rubber particles fuse into large masses in the cell. The tissue is from a cold-stimulated greenhouse-grown plant. Bar represents 2.4 µm. 12) Cold-stimulated basal parenchyma cells contain many rubber particles in addition to mitochondria, chloroplasts, and vesicles. Bar represents 6.32 µm.

present in the cell (Figure 8). Deposition continues until the cells become filled with rubber (Figure 9). Other studies have reported rubber particles in the cytoplasm of young tissue (11, 12). The recurrent association of rubber particles and cytoplasm suggests that the rubber is synthesized cytoplasmically (7, 8, 10). Rubber formation occurs concurrently with the usual cellular processes; therefore, the relationship between rubber formation and vacuolar ultrastructure and ontogeny is pertinent to an understanding of rubber formation in guayule. In meristematic tissue from greenhouse-grown plants, rubber particles often are surrounded by a thin layer of cytoplasm and are located between vacuoles (10). It is possible that rubber is produced cytoplasmically and that vacuoles then form, surrounding the cytoplasm and rubber particles (10). The appearance of rubber particles in the vacuolar regions of cells may be the result of tonoplast rupture, marked by membranous fragments and degraded-appearing cytoplasm (Figure 10). The tonoplast may weaken due to the action of enzymes released from smaller vacuoles during development of the parenchyma cells and due to the pressure of increasing numbers of rubber particles. Other investigators (13) also noted the lack of an intact tonoplast as well as the close association of rubber particles with the cytoplasm, which becomes disorganized as rubber particle number increases.

Rubber particle fusion has been noted by many investigators (7, 8, 12, 14). Particle fusion varies from two particles uniting, as revealed in Figure 9 (a micrograph from a field-grown cold-stimulated plant), to the coalescence of many particles into a large mass, as shown in Figure 11 (a micrograph from a greenhouse-grown cold-stimulated plant). An osmiophilic film surrounds rubber particles; however, it has not been proven whether the film is a true phospholipid and protein coat.

Cortical parenchyma cells from the base of cold-stimulated plants are large and filled with rubber particles, chloroplasts, mitochondria, and vesicles (Figure 12). Basal cortical parenchyma organelles such as the nucleus and chloroplast in Figure 13 appear to be functional. The intact and functional appearance of organelles in the base of guayule stems that had experi-

enced two winters has been noted (14). Even in the mature tissue from the plant base, rubber particles are associated with cytoplasm and cellular organelles and are surrounded by sealed vesicles (Figure 14). The proliferation of vesicles, associated with lytic enzymes and degraded cytoplasm, is considered part of the cold temperature phenomenon (11). Vesicles appear to arise in the cytoplasm from endoplasmic reticulum, dictyosomes, and dilation of the tonoplast (Figure 15). Vesicles similar in appearance to those in guayule have been observed in the laticiferous system of *Papaver somniferum* L. (15). Vesicles in the laticifers of *Papaver bracteatum* Lindl., which form from dilation of endoplasmic reticulum, may enlarge by the fusion of smaller vesicles (16). The release of enzymes from the fusion of smaller vesicles in guayule could account for the degraded-appearing cytoplasm.

Guayule rubber particle dimensions may provide clues to the mode of synthesis of rubber particles and to the behavior of rubber particles in the cells. Areas of rubber particles measured after cold stimulation averaged 1.49 μm^2, with a range of 0.04 μm^2 to 29.2 μm^2 (7). Another study reported sizes ranging from 0.1 to 1.5 μm (14), and another (12) observed spheres from 0.07 to 3.0 μm and masses from 5 to 10 μm. Rubber particle fusion would explain the wide variation in size. Rubber particles are manufactured and stored in the parenchyma cells and have no need of mobility afforded by small, membrane-bound particles. The absence of a membrane would facilitate rubber particle fusion, whereas a phospholipid and protein coat might impede fusion. The formation of rubber in stem parenchyma cells focuses attention on cellular organelles as possible sources of rubber precursors. Numerous mitochondria are located in both cold-stimulated and non-cold-stimulated tissue (10). The mitochondria provide a source of ATP, which supplies the large energy requirement of rubber biosynthesis.

Chloroplasts are frequent constituents of parenchymatous tissue (10, 14). The ability of guayule plants to fix CO_2 has been examined by following the uptake of $^{14}CO_2$ into rubber and determining the proportion of CO_2 fixed by leaves and stems (14). Bark chloroplasts do contribute to CO_2 fixation, and some of the fixation products are incorporated into rubber. The main source of photosynthesis remains the leaf tissue, with a contribution of 86.5 percent. The

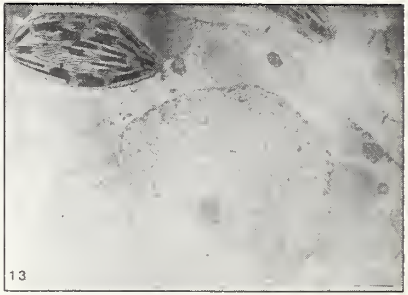

Fig. 13

Fig. 14

Figures 13-14. 13) The nucleus and chloroplast from this cold-stimulated parenchyma cell from the plant base appear to be intact and functional. Bar represents 1.5 μm. 14) Rubber particles are surrounded by sealed vesicles containing fibrillar material in this micrograph from cold-stimulated basal cortical parenchyma. Bar represents 1.3 μm.

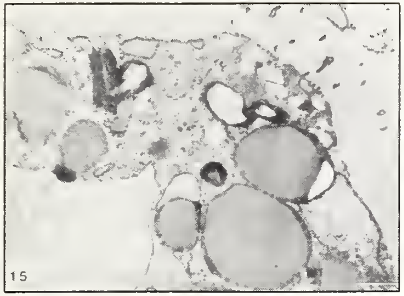

Fig. 15

Figure 15. Vesicles form from endoplasmic reticulum, dictyosomes, and dilation of tonoplast next to rubber particles in this micrograph from basal cold-stimulated parenchyma tissue. Bar represents 0.6 µm.

greater ability of leaves to fix CO_2 is attributed to the increased number of chloroplasts in the leaves (approximately 60 per cell in leaf tissue versus 30 per cell in stem tissue). The meristematic stem cells incorporated the least amount of ^{14}C into rubber, possibly because the photosynthates were needed for general metabolism. Cells in the oldest part of two-year-old plants were filled with rubber, yet ^{14}C continued to be incorporated into *cis*-polyisoprene (14). The functional appearance of chloroplasts in basal stem tissue would substantiate the continued biochemical activity (8, 10, 14).

Morphological and anatomical studies show that rubber content is an expression of the genetic makeup of guayule plants. Morphological features such as leaf shape and trichome morphology may be used to assess a plant's rubber-producing capability. High- or low-rubber-producing potential may in part be explained by anatomical analysis of parenchymatous tissue, the site of rubber biosynthesis and storage. Ultrastructural examination of cold-stimulated and non-cold-stimulated parenchyma cells shows that in order to respond to low-temperature stimulus cells must reach a certain degree of maturity. Rubber biosynthesis is cytoplasmic, and rubber particles fuse into large masses in the cells. Rubber formation in the guayule plant is a complex biochemical, structural, and environmental interaction. Research advances in the biochemistry and structure of the guayule plant should occur concurrently in order for a complete understanding of rubber biosynthesis and deposition to ensue.

ACKNOWLEDGMENTS

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Chapter 4

Genetics, Cytogenetics, and Breeding of Guayule

Ali Estilai and Dennis T. Ray

EARLY ATTEMPTS AT GUAYULE DOMESTICATION AND BREEDING

The commercial utilization of guayule dates back to 1888 when the New York Belting and Packing Company processed nearly 45,000 kg (100,000 lb) of shrub, imported from Mexico, and obtained high-quality natural rubber from guayule bark. Serious attempts at cultivation of guayule, however, began after the Mexican revolution in 1911 and the subsequent closure of the processing factories. The Intercontinental Rubber Company and two of its employees, Drs. F.E. Lloyd and W.B. McCallum, were responsible for the initial cultivation of guayule for rubber production (1-3). After 17 years of hard work, moving his project from Mexico to San Diego, California, then to Continental, Arizona, and finally back to California, Dr. McCallum succeeded in transferring the wild plant from its desert habitat to 3,300 ha (8,000 acres) of cultivated fields near Salinas, California. It has been reported (4, 5) that Dr. McCallum made as many as 1,300 different selections, one of which, variety 593, was planted extensively during the Emergency Rubber Project (ERP).

Dr. McCallum introduced a new industrial crop to southwestern agriculture. Unfortunately, he was not able to increase the rubber content of his selections beyond the wild state. The reproductive biology of guayule was not known at the time, and the selection procedures perpetuated the apomictic types that produced uniform progenies, identical to the female plant from which they were derived (4-6).

During the ERP, a large number of scientists became involved in basic research on guayule. The knowledge generated about chromosome number, self-incompatibility, and the mode of reproduction was essential to begin a successful plant breeding program in guayule. Unfortunately, the investigations were of short duration and did not contribute to the development of superior varieties which could have been profitable once the emergency situation was lifted. From 1946 to 1959, the plant breeding activities included evaluation of 25 selections, commonly known as the "USDA varieties," collection of bulk seed from those selections, and the deposition of the seed at the National Seed Storage Laboratory at Ft. Collins, Colorado. Hammond and Polhamus (1), and more recently Thompson and Ray (6), have reviewed the subject.

In the late 1970s, the renewed interest in developing guayule as a domestic source of natural rubber brought guayule plant breeding to the forefront of the research activities needed to bring about successful commercialization of the wild plant. Among the early pioneers who began plant breeding work by planting guayule seeds received from Ft. Collins and from new collections made in Mexico and Texas were Dr. G.P. Hanson and his colleagues at the Los Angeles State and County Arboretum (LASCA), Drs. H.M. Tysdal (a veteran post-World War II guayule breeder), I.A. Siddiqui (California Department of Food and Agriculture), P.F. Knowles (University of California, Davis), V.B. Youngner (University of California, Riverside), H.H. Naqvi (who joined the University of California, Riverside, from the LASCA project), D.D. Rubis, and D.L. Johnson (University of Arizona, Tucson), J.W. Whitworth (New Mexico State University, Las Cruces), and J.L. Tipton (Texas A&M, El Paso, Texas).

Today's plant breeding and genetics research is centered at the University of California, Riverside; the University of Arizona, Tucson; and the USDA Water Conservation Laboratory, Phoenix, Arizona. Besides the authors of this chapter, Drs. J.G. Waines, A.E. Thompson, D.A. Dierig, and A. Hashemi are involved in genetics, cytogenetics, and plant breeding research on

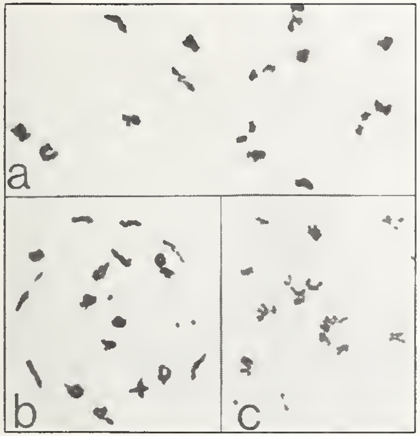

Figure 1. Meiotic chromosome pairing in a diploid plant with 2n = 36 (a), a diploid plant with 18 pairs and 3 B-chromosomes (b), and in a tetraploid guayule plant (c). (a and b, courtesy of Dr. Ahmad Hashemi, Department of Botany and Plant Sciences, University of California, Riverside.)

guayule. Basic research on molecular biology of guayule rubber biosynthesis began recently, and Dr. R.A. Backhaus (Arizona State University, Tempe) and Dr. K. Cornish (USDA/ARS) are active in that field. The University of California, Irvine, and the University of Arizona, Tucson, are also involved in molecular biology of rubber to a limited extent.

BREEDING SYSTEM AND CHROMOSOME NUMBER

Guayule is a member of the Asteraceae family. It flowers profusely, with each head carrying five female ray-florets (seed-producing) on the periphery and numerous male disk-florets (pollen-producing) in the center. The method by which a guayule plant produces seed (achenes, to be exact) depends on its genetic makeup. Studies by Bergner (7, 8) and Stebbins and Kodani (9) have shown that guayule plants may have 36, 54, 72, or higher number of chromosomes (Figure 1). These numbers constitute a polyploid series with the basic chromosome number of x = 18. In addition to the above normal or "A" chromosomes and their aneuploid variants, Catcheside (10) reported the presence of one to several very small chromosomes, referred to as "B" or supernumerary chromosomes, in some plants. Recent cytological observations have found "B" chromosomes not only in guayule (6) but also in *P. hispidum* Raf. var. *auriculatum* (Britt.) Rollins (11). No comprehensive study has been conducted to determine the adaptive role of "B" chromosomes in the genus *Parthenium*. Casual observations suggest no correlation between the morphology of a guayule plant and the presence or absence of "B" chromosomes.

Different chromosome numbers and the varied mode of reproduction make guayule a fascinating organism to study. With respect to breeding, however, guayule is a difficult organism to work with, and success in its improvement depends on a thorough understanding of its peculiarities, especially those presented in the sections that follow.

Sexual Reproduction and Self-Incompatibility

The mode of reproduction in diploid guayule plants with $2n = 36$ chromosomes is sexual. Diploids are outcrossers because of the inability of their pollen to grow on the surface of the stigma of their own flowers and to fertilize the eggs. The sporophytic system of incompatibility found in guayule is different from the gametophytic system found in tobacco and a number of other plants. A single locus with a series of R alleles determines the incompatibility relationships in guayule (12-14). In its most simplified form, the presence of the same R allele in the pollen and stigma will inhibit germination of the pollen grain or prevent the pollen tube from penetrating the stigmatic surface. In its complex form, success or failure of a cross in producing viable seeds not only depends on the genotype of the stigma in the female parent, but also on the dominance relationship of the multiple alleles in the pollen-producing parent. Studies of other *Parthenium* species have revealed a widespread occurrence of self-incompatibility alleles among most of the diploid species (11, 15-20). The incompatibility mechanism prevents selfing and maintains a high degree of heterozygosity in the populations needed for the long-term evolutionary adaptation of the species. The drawback of self-incompatibility in guayule has been the failure to produce homozygous genotypes. In some crops, it has been possible to find alleles for self-compatibility. No comprehensive study has been conducted to determine the presence of such alleles in guayule.

Apomixis

Naturally occurring triploid and tetraploid guayule plants reproduce by apomixis (21-25). Seeds produced by apomixis are an exact replica of the plant from which they were collected in regard to their chromosome number and genetic makeup. Defined as such, apomixis, or asexual seed formation, is comparable to vegetative or clonal propagation. Based on the origin of the

embryo sacs, the requirement of pollination, and other factors, several forms of apomixis are recognized (26), discussion of which is beyond the scope of this chapter. Apomixis in guayule is called "generative diplospory" because the embryo sacs are produced from unreduced megaspore mother cells (MMCs). The meiotic division, which normally produces reduced gametes with the haploid chromosome number, is blocked in the MMCs. As a result, the embryos have the same chromosome complement as the maternal plant. Also, apomixis is pseudogamous in guayule, which means pollination is required for the fertilization of the central nuclei for endosperm development. If all seeds are asexually produced, we speak of obligate apomixis. As such, apomixis is considered an evolutionary dead end for the species. Apomictic guayule plants produce a residual sexual seed by normal meiosis followed by fertilization. This mode of reproduction is known as facultative apomixis.

Based on the preceding discussion, a facultatively apomictic guayule plant may produce four types of seeds: asexual seeds, polyhaploid seeds derived from the parthenogenic development of reduced eggs without fertilization, sexual seeds whose chromosome number equals that of the reduced embryo plus the chromosome number of the male gamete, and sexual seeds whose chromosome number equals that of the unreduced embryo plus the chromosome number of the male gamete (1, 6). This explains the presence of the various off-types among the progenies of any individual apomictic plant. The sexual progenies are important sources of genetic variation from which selections for further improvement can be made. Polyhaploids, however, produce smaller amounts of biomass and have lower rubber yield. If a large number of those plants are found among the progenies of a selected variety, it will be difficult to show the true progress made in increasing the yield.

Several investigators (22, 23, 27-30) conducted limited research to determine the genetic basis of apomixis in guayule. Their conclusions are mostly speculative, leaving the explanation of the genetic control of apomixis confusing at best. The researchers, however, agree that only a small number of genes are involved. A minimum of three loci, one inhibiting meiosis in the

MMCs, another preventing fertilization, and the third controlling pseudogamous development of the seeds, is sufficient to explain apomixis in guayule. It was noted that apomixis in guayule is genic and not the immediate consequence of polyploidy (22, 27). Recent studies of Hashemi et al. (19, 31) on the mode of reproduction of artificially produced tetraploids confirm that conclusion.

Polyploidy

Polyploidy has extended the geographic distribution of guayule. Diploids are found only in a limited area near Mapimi in Durango, Mexico (1, 25). Polyploids, on the other hand, are found over a wide range, from north central Mexico to southwestern Texas. The abundance of triploids and tetraploids in the early collections, their ability to produce mostly uniform progenies, and their superiority to diploids in biomass, vigor, and disease resistance may explain why all of the 25 USDA selections as well as the standard variety 593 are exclusively polyploids.

The origin of polyploids is not known in guayule. Morphological similarities between diploids and polyploids and the cytological observation of some trivalents and tetravalents in polyploids suggest a possible autopolyploid origin in guayule (6). Polyploidy and apomixis have been responsible for the slow progress in the understanding of the genetic basis of rubber production in guayule. In addition, polyploidy has made it difficult to produce true breeding lines.

Literature suggests that apomictic polyploids are self-compatible and, thus, capable of fertilizing the central nuclei for production of endosperm with their own pollen. In a study by the senior author (unpublished), a number of polyploid plants failed to produce viable seeds upon selfing. The study clearly indicated that some of the naturally occurring polyploids were not self-compatible. Failure to produce seed by self-fertilization is not a serious problem if a selected variety is grown for rubber production. But, if the new variety is to be increased by

seed, provisions must be made for the presence of a compatible pollen donor in the area where the selected variety is being increased. Of course, another way of overcoming this problem is to develop self-compatible apomictic lines.

BREEDING OBJECTIVES FOR CULTIVAR DEVELOPMENT

A number of guayule traits must be modified before the plant can become a suitable crop for commercial production. Improving rubber yield per unit area of land, however, is the primary objective. Since natural rubber is used in specific applications, the quality of guayule rubber must meet the industry's standards. Therefore, the improvement of rubber quality is also an important goal. All other breeding objectives directly or indirectly affect the primary objective of improving rubber yield. Among these are regrowth ability for multiple harvest, reduced postharvest rubber degradation, processing quality of the shrub, and disease and insect resistance. Improvement in coproducts, seed size, seed germination, seed yield, resistance to herbicides, and tolerance to environmental stress, such as cold, drought, and salt, also need breeders' attention.

Rubber Yield

Guayule rubber yield is usually expressed as a product of dry matter per hectare and the mean rubber content:

$$Y_r = Y_{dm} R \qquad (1)$$

where Y_r is the yield of rubber, kg/ha; Y_{dm} is the yield of dry matter, kg/ha; and R is the mean rubber content of the dry matter, kg/kg. In different tests, plants may stay in the field for varied

lengths of time. For comparison purposes, it is more appropriate to express the yield on an annual basis:

$$Y_a = 12\ Y_m \tag{2}$$

and

$$Y_a = 365\ Y_d \tag{3}$$

where Y_a is annual rubber yield in kg/ha; Y_m is rubber yield in kg/(ha month); and Y_d is rubber yield in kg/(ha day).

The above equations show that rubber yield may be improved by increasing biomass, rubber content, or both. Of these two major yield components, improving the rubber content is more desirable because the increase in biomass involves additional costs associated with harvest, transportation, and processing of the extra shrub.

Rubber content. Breeding for increased rubber content has been frustrating because in many cases the progenies of individual selections have not repeated the high rubber content of the selected parents (32-34). In addition to genotype, the following factors affect the rubber content of guayule: edaphic and climatic factors, cultural practices, irrigation regimes, age, sampling date, and the proportions of bark and wood tissues in the plant samples used for analysis. The procedures of rubber content determination are also major sources of the variations reported in rubber content.

Based on the foregoing discussion, it is difficult to answer the often-asked question, "What is the maximum attainable rubber content?" In the native stands, rubber content has been reported to range from 3.6 to 22.8 percent (1, 35, 36). Higher values, up to 26 percent, have also been mentioned, but not confirmed. Under cultivation, however, rubber content has varied from 3 to 8 percent in recent tests (1, 6, 32-34, 37-44). It has been implied (1) that the higher rubber content in the native stands may be due to a plant's age which, in some cases, may reach

up to 40 years. This is an erroneous notion in light of recent studies which showed that as a plant's age increased, its bark/wood ratio decreased (41, 42). Since most of the rubber is in the bark, older plants are expected to have lower rubber content. In the Shafter yield tests, 24-month-old plants had higher rubber content than 45-month-old plants (44). Therefore, the high rubber contents reported for some native plants need other explanations. The edaphic and climatic factors and/or certain environmental stresses may be responsible for the high rubber contents. It is also possible that those high values were obtained by analyzing only the bark tissue. Also, the possibility of laboratory errors should not be overruled.

Biomass. Guayule plants also exhibit a great variation in biomass production (Table 1). In the Uniform Regional Variety Trials (URVT) and the yield tests of 28 different selections at Shafter, California, annual biomass yields of 12,700 to 27,800 kg/ha have been obtained. Increasing biomass does not appear to be a major problem. Variation within guayule itself and that generated from interspecific hybridization of guayule with several tree-like species provide ample opportunities to increase the biomass. The difficulty, however, is the negative correlation between biomass and rubber content, especially in the F1, F2, and backcross generations derived from interspecific hybridizations (5). Irrigation experiments have also shown that increased irrigation gives higher biomass with lower rubber content (1, 6, 45, 46). Fortunately, the negative relationship between biomass and rubber content is not absolute, and development of genetic recombinants that carry desirable genes for both increased rubber content and biomass is possible. The major task in producing those genotypes involves making numerous crosses and screening a large number of plants in the segregating generations.

Several studies have reported high positive correlations between biomass and rubber yield and no correlations between rubber content and rubber yield (47). However, to develop guayule as a crop, we must go beyond selection for increased biomass and the maintenance of the status quo for rubber content. Increasing rubber content must become the highest priority in future research.

Yield. Experiments conducted in the 1940s and early 1950s gave annual rubber yields of 220 to 560 kg/ha (1) which are comparable to the rubber yields of 216 to 570 kg/ha obtained in the first URVT. Considering that selections used in the first URVT represented seven of the old USDA varieties, the similarity in the yield performance is not surprising.

Table 2 presents rubber yield of 24 new selections and four old varieties, at age 21 and 45 months, under Shafter, California, field conditions. In these tests, several new selections outyielded the old check varieties. These studies put the potential annual yield of the best of the available guayule germplasm at 600 to 900 kg/ha. Two of the new germplasms (Cal-6 and Cal-7) were included in the second URVT, where they were compared to five USDA varieties and AZ-101 (Table 1). Cal-6 and Cal-7 ranked first and second at Riverside, California. Cal-7 was first at Ft. Stockton, Texas, and second at Maricopa, Arizona. Cal-6 ranked first at Las Cruces, New Mexico, and second at Maricopa, Arizona. Over all locations, Cal-6 was first and Cal-7 was second. Although Cal-6 and Cal-7 show some degree of plasticity, they are not the highest rubber producers in every location. This emphasizes the importance of the URVT in the evaluation of new selections prior to their release for commercial production.

More experimental data on the rubber yield is available from the irrigation experiments conducted in Arizona (45). Annual rubber yields of 380 to 660 kg/ha were obtained under different irrigation regimes for varieties 593, 11591, and N565-II. It is interesting to note that the three varieties used did not show any significant differences in their rubber yield under the six irrigation treatments.

In a recent study (6, 47) in which 423 plants were compared for their rubber yield, individuals with more than 125 grams of rubber per plant were found. Based on the yield of individual plants and assuming a population density of 27,500 plants/ha, calculations indicate that an annual rubber yield in excess of 1,100 kg/ha might be obtainable.

A new approach. As mentioned earlier, up to the present time improvements in rubber yield have been sought through the manipulation of the two major yield components—the plant's biomass and rubber content. Since biomass consists of two components, bark and wood, and

Table 1. Rubber and resin content, biomass, resin, and rubber yield of guayule entries in first and second Uniform Regional Variety Trials (URVT).

Entry	Content		Yield		
	Resin (%)	Rubber (%)	Biomass (kg/ha/y)	Resin (kg/ha/y)	Rubber (kg/ha/y)
First URVT, 1982-1985, over four states and nine locations[a]					
N565	7.6	7.3	4,450	338	340
N576	6.1	6.7	5,879	358	356
11591	6.0	6.6	4,944	296	314
11605	7.0	7.2	5,314	372	372
11619	6.3	7.0	5,222	329	341
12229	6.5	6.9	5,046	328	337
Second URVT, 1985-1988, data by lines and locations					
N576:					
Ft. Stockton, TX	9.1	8.6	3,764	341	322
Rio Grande City, TX	5.0	4.9	3,948	197	187
Uvalde, TX	7.1	3.2	8,823	628	287
Las Cruces, NM	4.8	5.6	3,552	168	198
Riverside, CA	7.2	4.6	8,662	627	395
11605:					
Ft. Stockton, TX	11.3	8.9	4,623	516	400
Rio Grande City, TX	5.6	4.0	5,496	311	217
Uvalde, TX	7.9	2.5	8,482	664	205
Las Cruces, NM	6.0	6.1	3,614	216	217
Riverside, CA	8.3	5.0	11,533	967	572

Table 1. (continued).

Entry	Content: Resin (%)	Content: Rubber (%)	Yield: Biomass (kg/ha/y)	Yield: Resin (kg/ha/y)	Yield: Rubber (kg/ha/y)
N396:					
Ft. Stockton, TX	9.3	8.4	4,254	395	360
Rio Grande City, TX	5.8	5.7	3,804	222	216
Uvalde, TX	—	—	—	—	—
Las Cruces, NM	4.5	5.4	5,180	228	270
Riverside, CA	7.7	5.0	11,652	899	577
Cal-6 (250):					
Ft. Stockton, TX	10.2	8.2	5,490	556	450
Rio Grande City, TX	5.1	4.9	6,552	337	324
Uvalde, TX	8.4	3.2	8,292	694	267
Las Cruces, NM	5.1	6.8	4,879	249	327
Riverside, CA	9.4	6.8	13,798	1,296	938
Cal-7 (254):					
Ft. Stockton, TX	9.9	8.0	6,397	678	503
Rio Grande City, TX	5.5	5.2	5,388	304	274
Uvalde, TX	8.4	3.8	7,484	622	279
Las Cruces, NM	5.1	4.7	5,041	254	239
Riverside, CA	9.0	6.4	13,753	1,227	887

Table 1. (continued).

Entry	Content		Yield		
	Resin (%)	Rubber (%)	Biomass (kg/ha/y)	Resin (kg/ha/y)	Rubber (kg/ha/y)
AZ-101:					
Ft. Stockton, TX	10.8	2.7	6,650	716	180
Rio Grande City, TX	6.8	2.7	4,932	338	142
Uvalde, TX	—	—	—	—	—
Las Cruces, NM	5.5	3.1	4,432	242	137
Riverside, CA	8.7	2.2	19,028	1,656	412
11604:					
Ft. Stockton, TX	10.0	7.5	5,971	595	449
Rio Grande City, TX	5.6	3.6	5,640	316	209
Uvalde, TX	—	—	—	—	—
Las Cruces, NM	5.3	4.5	4,719	252	209
Riverside, CA	9.0	5.5	13,336	1,196	738
11634:					
Ft. Stockton, TX	11.5	8.0	5,027	577	402
Rio Grande City, TX	5.5	6.1	7,080	396	439
Uvalde, TX	8.8	2.7	6,168	538	163
Las Cruces, NM	5.0	5.1	4,089	206	208
Riverside, CA	9.9	5.9	12,098	1,193	711

Table 1. (continued).

Entry	Content		Yield		
	Resin (%)	Rubber (%)	Biomass (kg/ha/y)	Resin (kg/ha/y)	Rubber (kg/ha/y)
Second URVT, 1985-1988, data by lines over five locations					
N576	6.7	5.4	5,845	402	277
11605	7.8	5.3	6,750	534	322
N396	6.8	6.1	6,222	436	356
Cal-6 (C250)	7.9	6.0	7,941	658	461
Cal-7 (C254)	8.0	5.7	8,045	667	436
AZ101	8.5	2.6	9,760	853	217
11604	7.5	5.3	7,417	590	401
11634	8.5	5.6	7,228	624	384

[a]See reference 37 for detailed information.

since the rubber content is significantly different in those tissues, the senior author has suggested using four components to express guayule rubber yield:

$$Y_r = Y_b R_b + Y_w R_w \qquad (4)$$

where Y_r is rubber yield, kg/ha; Y_b is bark dry matter, kg/ha; R_b is rubber content of the bark, kg/kg; Y_w is wood dry matter, kg/ha; and R_w is rubber content of the wood. Research has begun to determine the level of genetic variability for those components and subsequently to

Table 2. Rubber and resin content, annual dry matter, rubber, and resin yields of 21- and 45-month-old guayule selections at Shafter, California.

Guayule selections	Rubber content (%)		Resin content (%)		Ann. dry matter (kg/ha)		Ann. rubber yield (hg/ha)		Ann. resin yield (kg/ha)	
	21	45	21	45	21	45	21	45	21	45
Cal-6	7.75	5.64	7.50	8.01	11,817	14,015	908	796	879	1,124
Cal-7	7.18	5.66	7.27	8.38	9,497	12,360	680	687	694	1,038
C271	7.08	4.41	7.03	7.61	11,090	12,030	778	612	775	1,059
C255	6.61	5.47	7.20	8.16	10,840	15,080	710	826	779	1,231
C245	6.78	5.24	7.18	8.23	10,026	15,860	683	828	716	1,305
C215	7.08	4.87	7.60	7.88	9,694	14,445	682	701	736	1,135
C211	7.85	5.64	8.03	7.83	9,524	12,880	619	726	626	1,009
C288	6.37	5.43	6.96	7.02	8,615	10,455	549	560	594	730
N565	7.11	4.42	6.83	7.82	5,623	7,950	400	348	375	616
C355	5.20	4.08	8.21	7.35	7,135	12,070	359	493	568	888
593	5.69	4.22	5.05	4.55	5,743	8,720	325	362	290	384
11591		4.46		5.54		8,550		374		470
11605		4.38		7.21		10,660		468		774
C210		5.03		6.20		8,820		441		545
C212		4.66		6.30		10,500		492		663
C219		4.30		6.46		11,710		504		756
C221		4.24		6.16		9,570		401		584
C222		4.49		5.88		8,630		385		506
C225		5.40		6.61		10,240		551		664

Table 2. (continued).

	Rubber content (%)		Resin content (%)		Ann. Dry matter (kg/ha)		Ann. rubber yield (kg/ha)		Ann. resin yield (kg/ha)	
Guayule selections	21	45	21	45	21	45	21	45	21	45
C226		4.54		7.95		15,220		690		1,210
C230		4.41		4.04		7,490		330		301
C235		4.42		5.54		10,340		451		572
C249		5.06		7.60		11,695		595		885
C270		4.06		6.22		14,780		602		919
C275		5.41		8.06		12,140		658		980
C297		4.77		6.61		10,250		486		672
C354		2.28		5.77		26,110		606		1,500
C362		4.31		8.42		12,150		516		1,009

cross appropriate genotypes to produce new genetic recombinants. In a preliminary study, a range of 0.55 to 1.16 was found for bark/wood ratio for 15 different germplasms (42). This shows that a considerable variation exists for the components of biomass in guayule. Maximizing bark, by developing plants that partition their total biomass toward production of more bark and less wood, has become an important goal of future research. Expressing rubber yield in terms of four components is expected to refine the genetic manipulation of each component independently and in relation to each other. For example, a genotype with high bark/wood ratio but low bark rubber content may be crossed with another genotype with high bark rubber content in order to combine the desirable genes from two parents in the hybrids. It should be

emphasized that, in the yield tests, rubber yield will always be obtained from the two major components, total biomass and plant's rubber content.

Rubber Quality

It has been reported (2) that the quality of guayule rubber is very similar to that of *Hevea* rubber and that guayule rubber may be used for similar applications as *Hevea* rubber.

Rubber quality is not well defined for guayule. Because it is a long polymer, the physical and processing properties of rubber are greatly influenced by the molecular weight (MW) and molecular-weight distribution (MWD). In guayule, weight-average molecular weight ($\overline{M}_w$) number-average molecular weight ($\overline{M}_n$), and polydispersity value ($d = \overline{M}_w/\overline{M}_n$) are commonly used to partially determine rubber quality (48). Values of 1×10^5 to 2.5×10^6 and 6×10^4 to 8×10^5 have been reported for $\overline{M}_w$ and $\overline{M}_n$, respectively (49, 50). The variations reflect genetic, environmental, seasonal, and age differences of the guayule materials used by the investigators mentioned earlier in the chapter. Differences in the procedures of molecular-weight determination may also be responsible for part of the observed variation. A recent study has shown that guayule tissue is also a major source of variation (50). In the study, bark and wood were compared for their rubber molecular weight. In all of the genotypes studied, the bark tissue consistently contained rubber with higher $\overline{M}_w$ and $\overline{M}_n$. On average, $\overline{M}_w$ of bark rubber was twice the $\overline{M}_w$ of wood rubber. Based on this study, every guayule genotype is capable of yielding three qualities of rubber—high (from the bark), low (from the wood), and intermediate (from the whole shrub).

Early plant breeding works assumed that any type of guayule would produce acceptable rubber and, therefore, were not concerned with rubber quality. The observation of great variation within guayule and the results from interspecific hybridizations have changed our views regarding rubber quality. The disappointing failure of 'Gila-1' to produce rubber with acceptable quality has also served as a warning to include rubber quality as an important part of

plant breeding activities. With the availability of gel permeation chromatography for molecular weight determination, monitoring rubber quality has become part of the current plant breeding work.

The genetic basis of rubber quality within *Parthenium argentatum* Gray has not been investigated. The interspecific hybridization, however, has provided some information that must be interpreted with extreme caution. F_1 plants from crosses of *P. schottii* Greenm. ex Millspaugh & Chase ($\bar{M}_w$ = 2 x 10^3) and guayule ($\bar{M}_w$ = 2 x 10^6) showed two peaks, one for each parental $\bar{M}_w$. This was interpreted as a codominant relationship between the alleles of the two species (18). Rubber of the F_1 plants from crosses of guayule ($\bar{M}_w$ = 1 x 10^6) and *P. fruticosum* Less. ($\bar{M}_w$ = 4 x 10^4) had $\bar{M}_w$ of one million. This was interpreted as a dominant relationship between the alleles of the two species (51). In another report (52), hybrids from crosses of guayule ($\bar{M}_w$ = 2 million) and *P. tomentosum* DC. ($\bar{M}_w$ = 10,000) showed $\bar{M}_w$ of one million, suggesting an additive relationship between the alleles of the two parents. Further research is needed to determine if the above modes of gene action represent true genetic mechanisms involved in determination of rubber molecular weight or if they reflect the limitations of those studies.

Improvement in rubber quality not only requires genetic variability but also is extremely dependent on the accuracy of the techniques used to evaluate that variability. Since genotype, plant tissue, age, environment, and sampling time affect rubber $\bar{M}_w$, it is critical to develop standardized procedures so that values obtained by different laboratories may be compared.

Multiple Harvest

With the price of natural rubber constantly changing at the global level, future guayule farmers are not likely to keep the crop in the field for four or five years to distribute the prohibitive cost of stand establishment across several years of plant growth. It is generally agreed that economical direct seeding and/or considerable reduction in the cost of production of guayule seedlings

is needed for successful commercialization. Even if those goals are achieved, guayule cultivars with multiple harvest capability may be the key to economical commercialization.

Although a number of clipping experiments were conducted in the 1940s, 1950s, and 1980s (see 1, 6), only limited information on regrowth variation and its genetic basis is available. In one study mariola (*P. incanum* H.B.K.) and two USDA varieties, A48118 and N396, were compared. Mariola was found to be superior to the USDA varieties, both when harvested at ground level and when clipped at 15 cm above ground. It was suggested that the ability to regrow vigorously after harvest may be introduced into guayule through interspecific hybridization. The study also showed that the survival rate of A48118 was three times higher than that of N396, suggesting genetic variation that may be amenable to selection (53).

A three-year regrowth study at Shafter, California (40, 44), a two-year study at Riverside and Palmdale, California, and a joint study with the University of Arizona and the USDA Water Conservation Laboratory, Phoenix, Arizona, (unpublished) have shown that guayule genotypes respond differently to harvest at ground level. The survival rate of 28 selections cut at ground level, in February-March, ranged from 11 to 100 percent. The same selections responded poorly when harvested in May, the period of active growth. These results are in agreement with those reported by Hunter (54) who found the highest survival rate for the plants clipped during dormancy.

The observation of genetic variability for regrowth is encouraging. The incorporation of this desirable trait into guayule cultivars with high rubber yield is the next important step. Cultivars that could be harvested in two-year intervals are expected to eliminate the need for replanting after each harvest. They will reduce the time that guayule growers must wait for a financial return from the crop and will produce the same quality rubber in successive harvests.

Reduced Postharvest Rubber Degradation

After harvest, the guayule shrub is air dried in the field, baled, transferred to the processing plant, and stored for varied lengths of time prior to processing. These steps may take several weeks to several months, during which time substantial losses in rubber quantity and quality may occur. Taylor and Chubb (55, 56) compared fresh and stored bulk shrub of the standard variety 593 for rubber quality. They found that the fresh shrub, which was processed with a minimum delay after harvest, yielded the best rubber with the highest MW. On the other hand, guayule shrub that was field cured for a week, bailed with its leaves on, and stored for six weeks gave the lowest rubber quality. In another study (48), coarsely ground shrub samples stored at different temperatures showed rapid rubber degradation at 21°, 32°, and 49°C. In a three-year collaborative study with G.E. Hamerstrand, the senior author compared different guayule selections for the degree and the rapidity of postharvest rubber degradation under Shafter, California, field conditions (57). Three distinct patterns of change were observed for the rubber content (RC) and rubber molecular weight (RMW). One group of selections showed a gradual decrease in RC and RMW after four weeks of field storage. In this group, the RC and RMW of plants stored for four weeks were significantly lower than those of the fresh plants. In the second group, RC increased during the first two weeks and decreased during the next two weeks of storage. It is possible that in this group the enzyme machinery of rubber biosynthesis remained active, allowing the harvested shrub to continue to make rubber for a short period of time. In the third group, changes in rubber quality and quantity were minimal. Statistical analyses showed that the RC of fresh plants and of those stored for four weeks was not significantly different. More research is needed to determine the causes of degradation and to find out the genetic basis of delayed rubber degradation observed in some plants. More genotypes must be exposed to more storage conditions in order to screen those that may withstand the adverse effects of long storage.

Disease and Insect Resistance

In 1982 approximately 80 percent of the sexually-reproducing diploid guayule plants growing in a field near McFarland, California, were killed by the *Verticillium* wilt pathogen (*Verticillium dahliae* Kleb). The field had been under cotton production for many years before it was used to plant different guayule germplasms for research purposes. Those who visited the field commented on the devastating effect of the disease "as a blessing in disguise" and were unanimous in stating that "it is better to see such a thing now rather than later when farmers grow guayule for profit." Clearly, breeding for disease resistance commands major attention in every guayule improvement program and, for this reason, a chapter of this book has been dedicated to disease and pest problems (see Chapter 8).

In the same field, many guayule triploids and tetraploids, as well as some of the F_1, F_2, and backcross plants derived from crosses of guayule with *P. fruticosum* and *P. tomentosum*, survived the disease. This suggested the presence of a great reservoir of genes for disease resistance in guayule, as had been observed by other workers (1, 6, 58). Since 1983, the California project has been collaborating with Dr. S.M. Alcorn of the University of Arizona in the area of developing *Verticillium* wilt-resistant cultivars. Seeds of promising selections have been sent to Arizona where the plants have been screened for resistance to wilt by repeated inoculations. Plants that survived testings have been returned to California and subsequently have been crossed with other desirable selections. Screening and breeding for disease resistance is also an important part of the Arizona program (59). The genetic basis of resistance to *Verticillium* wilt and other guayule diseases has not been investigated. Such studies are badly needed if progress is to be made in developing disease-resistant cultivars.

Although a number of insects attack guayule (see Chapter 8), the damage from insects has not been considered serious. To our knowledge, no genetic and plant breeding research on guayule insect resistance is being conducted at the present time.

Environmental Stress

Cold-, salt-, and drought-tolerant cultivars are expected to expand the area of guayule cultivation to cooler climates, to semiarid lands, and to marginal areas.

Cold tolerance. Native stands withstand temperature fluctuations of -18° to 49° C which suggests that guayule has a natural ability to survive lower temperatures. Under cultivation, however, guayule has not been tested for cold tolerance in extremely cold environments. In the experiments conducted by Mitchell (60), seedlings and mature plants suffered severe injuries when exposed to temperatures of -7° to -10° C for several hours. Acclimatization of the plants, by gradual exposure to low temperatures, improved survival. These experiments also suggested a possible heritable difference between line A-5058 and variety 593. In a recent study, differential survival was observed for several selections under Palmdale, California, winter conditions (34).

Cold tolerance may also be introduced into guayule by interspecific hybridization. At least four *Parthenium* species (*P. incanum*, *P. alpinum* (Nutt.) T. & G., *P. hispidum*, and *P. integrifolium* L.) are known to be more cold tolerant than guayule. Crosses between guayule and these species have been successful, and F_1 and BC_1 (first generation backcross) plants have been produced (11, 16, 17, 19, 49). However, the number of plants obtained from the interspecific hybridization has been very limited, thus precluding testing of the hybrid progenies for their cold tolerance.

Drought tolerance. Many experiments have been conducted to determine the water requirements of guayule under cultivation (see 1 and 6 for review). These experiments have shown that although guayule may be considered drought tolerant, the productivity of the plant increases consistently with increased irrigation. The emphasis of the current plant breeding programs has been on the development of guayule cultivars that may compete successfully with irrigated crops. This choice has been unavoidable due to the lack of adequate funding to carry out breeding research on every front.

Salt tolerance. Experimental works of the 1940s, reviewed by Hammond and Polhamus (1), and of the 1980s, reviewed by Thompson and Ray (6), have considered guayule to be only slightly tolerant to soil salinity (see also Chapter 7). To our knowledge, no genetic variability for salt-tolerance has been reported, and no plant breeding activity is under way to develop salt-tolerant cultivars.

Seed Quality

Many aspects of guayule seed, including development of machinery for harvesting and cleaning seed, breaking the seed dormancy, treating seed for improved germination and seedling vigor, and repeated testing for direct seeding, have been addressed during the last decade (see reference 6 for review). On the other hand, no genetic studies have been conducted to identify genotypes with increased seed quality and seed germination. In a preliminary study by the senior author (unpublished) in which diploid and polyploid plants were compared for their seed size, the weight of 1,000 filled seeds ranged from 0.691 to 0.931 g for the 10 genotypes studied. As was expected, seed size was the smallest in diploids. Differences in seed size were also observed among polyploid plants, which may suggest a possible genetic variation amenable to selection and breeding. The genetic aspects of seed dormancy, seed size, and seed quality need attention in future research.

Coproducts

It is generally agreed that economical utilization of guayule coproducts, such as resins, bagasse, and waxes, may generate additional revenues to make guayule a successful commercial crop. Depending on the selection and the age of the plants at harvest time, guayule may produce as much or up to twice as much resin as it produces rubber (Table 2). Ideally, guayule plants that

could produce more rubber at the expense of resins will be the most suitable for development of commercial cultivars. At present, no such guayule plants have been identified.

Guayule selections produce different quantities and qualities of bagasse. As new uses are found for bagasse, guayule cultivars may be developed to accommodate the new demands.

It should be emphasized that guayule must be developed as a source of rubber. Genetics, breeding, and application research on coproducts are justified only if the coproducts are to provide additional income for guayule as a rubber crop.

SOURCES OF GENETIC VARIABILITY

Without adequate genetic variation for desired characteristics, little progress can be made in improving guayule beyond its wild state. As discussed earlier, both chromosomal and genetic variations are available in guayule. The genetic variation for any trait may be obtained from natural populations, through genetic recombination, from interspecific hybridization, by induced mutations, and from new techniques of molecular biology, where, in theory, genes from any living organism may be utilized in improving other organisms. This section is concerned with sources of variations within guayule and its close relatives.

Collections from Texas and Mexico

The available guayule germplasms stem back to the collections made in Mexico and Texas (see Figure 1, Chapter 2). Hammond and Polhamus (1) and recently Thompson and Ray (6) have reviewed the subject. The latest collection, with the specific objective of expanding the genetic base of diploid guayule, was made in 1986 from an area of approximately 2,500 km^2 near Mapimi, in Durango, Mexico. Those plants are currently under evaluation at Riverside, Califor-

nia. The highest rubber producers identified in this evaluation will be added to the diploid materials of the recurrent selection program.

McCallum Selections and the USDA Varieties

Of all of the selections made by McCallum, only the variety 593 has been used by the current breeding projects. According to Hammond and Polhamus (1), a total of 11,970 kg seeds of variety 593 were stored in airtight metal containers in 1952 when the California seed stockpiling program came to a conclusion. Variety 593 was a poor rubber producer in recent tests (Table 2). It is highly apomictic and uniform, which may explain its popularity in the 1940s.

From the 1942 collection, the ERP research, and Hammond's 1948 collection, 25 lines were developed which are commonly referred to as "USDA varieties." Some of these were tested in the 1950s at Shafter and Salinas, California, and in Texas. Since the USDA varieties were the primary source of guayule germplasm available to the breeders in the late 1970s, several investigators have carried out research evaluating their productivity (6, 32, 33, 37). As recognized by Hammond and Polhamus (1) and reemphasized by Thompson and Ray (6), the USDA varieties represent a narrow genetic base. The state of Durango, Mexico, provided germplasm for 21 of these varieties, and 15 of the varieties are descendants of collection #4265, which consisted of five plants selected in one location.

Recent Improved Germplasms

With the reestablishment of guayule plant breeding research in the late 1970s, attempts were made to produce seeds of several promising USDA varieties for immediate use. Varieties 11591, 11604, 11605, 11619, 12229, N565, and N576 were planted in 1980 and, after the removal of the obvious off-types, the above varieties were jointly released by the USDA Agricultural Research

Service and the Agricultural Experiment Stations of Arizona, California, New Mexico, and Texas (61).

The plant breeding programs have released and/or are in the process of releasing new germplasms for various needs. The California project has released seven germplasms (Table 3), three from interspecific hybridization, two from diploids, and two from apomictic polyploids (38, 62, 63). Six additional selections have been planted in New Mexico for seed increase and early evaluation. Another 30 selections are being evaluated at the time of writing (in a joint genotype-environment interaction study with the University of Arizona and the USDA Water Conservation Laboratory, Phoenix, Arizona) at Riverside and Palmdale, California, and Maricopa, Arizona. AZ-101 or its equivalent, Gila-1, has not been formally released. Like Cal-5 (which is derived from crosses between guayule and *P. tomentosum*), AZ-101 is vigorous and a high biomass producer with low rubber content. Although it is not suitable for commercial production in its current state, it is a valuable source for breeding guayule through a backcrossing program.

Related Species

The 16 related *Parthenium* species (see Chapter 2) are important sources for introducing desirable variability into guayule. The major drawback in improving agronomic traits of guayule through interspecific hybridization is that several generations of backcrossing are needed to improve rubber content and to select against undesirable characteristics. However, if the related species are the only sources for the traits of interest, the use of related species in improving guayule is well justified.

Table 3. Origin and some characteristics of guayule germplasms released in the 1980s.

PI no.	Designation	Origin and characteristics
478640	11591[a]	A selection from 4264-I, Powers, McCallum and Olson collection.
478640	11604	A selection from 4264-I, Powers, McCallum and Olson collection.
478643	11605	A selection from 4264-I, Powers, McCallum and Olson collection.
478645	11619	A selection from 4264-I, Powers, McCallum and Olson collection.
478652	12229	A putative hybrid.
478655	N565	A selection from 4264-I, Powers, McCallum and Olson collection.
478659	N576	A selection from Hammond and Hinton collection.
478666	Cal-1[b]	Open-pollinated seeds from F_2 and BC_1 plants derived from guayule. x *P. tomentosum*. Source of vigor, biomass, and resistance to *Verticillium* wilt.
478667	Cal-2	Open-pollinated seeds from F_2 and BC_1 plants derived from guayule x *P. fruticosum*. Source of vigor, biomass, and resistance to *Verticillium* wilt.
478664	Cal-3	Open-pollinated seed from diploid plants with 36 chromosomes.
478665	Cal-4	Open-pollinated seed from *Verticillium* wilt-resistant diploid plants.
	Cal-5	Open-pollinated seed from 204 F_2 and BC_1 plants derived from guayule x *P. tomentosum*. Source of vigor, biomass, *Verticillium* wilt resistance, and rubber production.

Table 3. (continued).

PI no.	Designation	Origin and characteristics
	Cal-6 (C250-3)	A tetraploid apomictic selection from "Bulk Arboretum." A high-rubber-yielding germplasm that outyielded 593, N565, 11605, and 11591 at Shafter, California (see Table 2).
	Cal-7 (C254-3)	A triploid selection from "Mexican Bulk." A high-rubber-yielding germplasm that outyielded 593, N565, 11605, and 11591 at Shafter, California (see Table 2).

[a]The first seven entries are from the old USDA varieties, re-released in 1983 (see reference 61 for details).
[b]See references 36, 62, and 63 for details.

PLANT BREEDING APPROACHES

A number of complimentary approaches have been used to improve guayule. The following is a brief discussion of the approaches as they relate to the reproductive modes and chromosome numbers of guayule.

Selection Among Apomictic Polyploids

This simple approach has been most widely used in breeding guayule. Since apomixis produces progenies that are, to a large extent, identical to the maternal plant, selection among apomictic plants for heritable traits of interest promises a moderate gain in a relatively short period of time. The degree of success, however, depends on the level of heterogeneity of the materials and on the number of plants that could be screened effectively. Standard variety 593, most of

the USDA varieties, Cal-6 and Cal-7 germplasms, and a number of other selections currently being evaluated in Arizona and California are apomictic polyploids produced by the above approach. Selection among apomictic plants has identified genotypes with regrowth potential, disease tolerance, increased bark/wood ratio, and delayed postharvest rubber degradation and has been effective in increasing biomass and rubber yield. This approach has not been effective in improving the rubber content, which may be due to the low heritability of this trait and the strong environmental influences on rubber biosynthesis.

Hybridization of Selected Apomictic Polyploids

The limited sexual reproduction of apomictic plants may be used to develop genetic recombinants by crossing the most promising apomictic selections. Controlled crossing is labor intensive and results in a small number of hybrid progenies. In addition, hybrids and apomictic progenies are not usually distinguishable at early stages of plant development due to the lack of genetic markers. These difficulties may explain the slow progress made in using this approach. To date, a number of crosses have been made with the selected California germplasms. The progenies are several months old and will not be available for evaluation for two more years. Genetic markers that can express themselves at the seedling stage are expected to increase the efficiency of this approach. In addition to diallelic crosses of selected plants, the promising selections are being planted together in a crossing plot in order to maximize production of hybrid progenies for further selection.

Recurrent Selection of Sexual Diploids

During the ERP, sexually reproducing diploids with $2n = 36$ were used only on a small scale, primarily in crosses with other *Parthenium* species. The confinement of diploids to a small area near Mapimi in Durango, Mexico, has been a major factor in their limited representation in the

various collections. Highly variable progenies, as well as comparatively smaller size, must have discouraged early breeders from using sexual plants in breeding guayule. Another difficulty with diploids is their self-incompatibility which prevents production of inbred lines by self-fertilization. In spite of those problems, sexual diploids provide an excellent opportunity to improve guayule in long-term programs by increasing the frequency of the desirable genes and bringing together genes from diverse sources (5).

The California breeding program uses a modified recurrent selection method to improve guayule at the diploid level. Initially, plants with 36 chromosomes were identified cytologically and analyzed for rubber content at the age of 10 months, and those with the highest rubber content (1.49-3.54 percent) were used to establish a base population. From this base population one germplasm, Cal-3, was released for use by other guayule breeders (62). Open-pollinated seed from the base population growing in an isolation plot was used to establish the first generation of the recurrent selection at Shafter, California. Seeds obtained from this plot were planted at Moreno Valley, California, to establish the second generation. Approximately 100 plants from this generation were analyzed for rubber content during January and February 1988. The rubber content ranged from 3.03 to 10.30 percent, showing a great variability for rubber content in the second generation of recurrent selection.

After a few more generations of selection and natural crossing of the selected individuals, the products of the recurrent selection program may be used in the following ways: a) if the biomass production of the selected population is economically acceptable, the seed from the whole population will be released as an open-pollinated sexual variety with high mean rubber content; b) if, during the course of the study, promising individuals are found in the population, their open-pollinated seed will be increased to release new diploid lines; c) if the selected diploids remain high in rubber content but small in size, they will be crossed to selected apomictic polyploids to increase their biomass (which also introduces apomixis and production of lines with more uniform progenies); and d) the chromosome number of improved diploids will be doubled artificially to produce tetraploid varieties.

As mentioned earlier, the genetic base of the diploid materials used to begin the recurrent selection program was narrow. To broaden this base, a collection of diploid plants was made in Durango, Mexico, in 1986. After evaluation, the most promising plants from this collection will be included in the ongoing recurrent selection program (64).

Autotetraploid Cultivars from Selected Diploids

After improving the traits of interest at the diploid level (where sexual reproduction and self-incompatibility allow crossing of the selected parents), the chromosome number of the improved diploids may be doubled to produce autotetraploid cultivars. In a recent study (31), seedlings of the Cal-3 diploid germplasm were treated with different concentrations of colchicine solutions for different lengths of time. Colchicine concentration of 0.125 percent applied for eight hours produced the maximum number of tetraploid plants. As had been reported earlier (13), the mode of reproduction of the induced tetraploids was found to be sexual. The raw tetraploids, like their diploid progenitors, were self-incompatible. The induced tetraploids had larger stomata, capitula, achenes, pollen, and leaves. The study showed that artificial production of autotetraploids from selected diploids could be accomplished with no difficulties.

Interspecific Hybridization and Backcrossing

The importance of interspecific hybridization in transferring traits of interest such as the ability to regrow, increased biomass production, and cold-, disease-, and drought-tolerance was discussed earlier and will not be repeated here. Although several germplasms are produced and released through interspecific hybridization, no acceptable variety is available yet. No large-scale comparisons for rubber yield and rubber quality have been made between guayule and the new interspecific germplasms. In a limited study, a mixture of two-year-old F_2 and BC_1 plants derived from crosses between guayule and *P. tomentosum* DC. var. *stramonium* (Greene)

Rollins averaged 2.86 percent in rubber content and 67,460 kg/ha in dry weight, giving an annual rubber yield of approximately 965 kg/ha. Although this is an impressive yield, the new germplasm is not yet acceptable for commercial production due to the costs associated with harvesting, transporting, and processing all of the low-rubber-bearing shrub. In addition, the rubber quality of the new germplasm may need to be improved. The results, however, show the power of interspecific hybridization in increasing rubber yield. If the rubber content of this interspecifc germplasm could be doubled in a rigorous long-term backcrossing program, interspecific hybridization would make its mark in the development of guayule as a domestic rubber crop.

Tissue and Anther Culture

During the last decade, tissue culture techniques have been developed for guayule asexual propagation (65-67). A number of USDA varieties have been tested for their ability to produce whole plants through tissue culture. Tissue culture not only provides a valuable medium to study rubber biosynthesis in a controlled environment, it also allows production of a large number of genetically identical clones from selected plants. The junior author has suggested the use of tissue culture to maintain the genetic stocks of selected guayule germplasms and cultivars. This suggestion is based on the fact that the identity of selected apomictic lines cannot be maintained in successive generations because of the complex breeding system, which involves both sexual and apomictic modes of reproduction (68).

In some crops production of haploids by anther and/or pollen culture and subsequent chromosome doubling of the haploid plants have been used to produce homozygous plants, which are badly needed for genetic analyses of desirable traits. The limited efforts to produce haploid guayule by means of anther culture have not been successful (69). In view of the great value of homozygous genotypes to the breeding programs, more research should be carried out

toward production of haploids in guayule. Culturing isolated pollen instead of whole anthers should be tested in future research.

Genetic Engineering

Basic research on the molecular biology of rubber biosynthesis is a challenging endeavor which may result in increased productivity of guayule in the future. With respect to the current plant breeding needs, however, genetic engineering does not appear to offer any immediate solutions, especially considering the present status of biochemical, physiological, and genetic knowledge of rubber production in guayule.

Currently molecular genetic research is conducted primarily at Arizona State University and the USDA/ARS, and, to some extent, at the University of California, Irvine, and the University of Arizona. The primary goal of genetic engineering research is to introduce foreign promoter genes into guayule and reconstruct the genome in such a way that the activity of the genes that control the production of enzymes involved in rubber synthesis is enhanced considerably. Attempts are being made to isolate and clone the genes responsible for production of rubber transferase, farnesyl transferase, and the protein that surrounds the rubber particles (70, 71).

GENETIC MARKERS FOR CULTIVAR IDENTIFICATION

The importance of an adequate number of easily recognizable genetic markers in guayule plant breeding, genetics, and cytogenetics research cannot be overemphasized. Because of polyploidy, apomixis, self-incompatibility, and, above all, the need for several years to conclude genetic studies, only one marker has been genetically analyzed in guayule. Future research must identify and genetically analyze morphological, isozyme, and restriction fragment length polymorphism (RFLP) markers.

Morphological Markers

Although a number of morphological variants for leaf color, leaf shape, growth habit, and plant height are often observed in every guayule field, no attempts have been made to study the inheritance of those traits.

Flower color was reported to be white for the entire *Parthenium* genus. In a search for other flower colors among diploids, polyploids, and progenies of interspecific hybrids, plants with purple flower color were found and subsequently were crossed with those having white flower color (14). Genetic analyses showed that purple flower color is recessive to white, and that at the diploid level only one gene-pair controls flower color. It was also noted that one of the USDA varieties, N565, may be identified on the basis of flower color because more than 50 percent of its individuals show purple flower color. Once the inheritance of purple flower color is studied at the polyploid level, this trait may be used to determine the degree of apomixis in guayule. It should be noted that purple flower color is not expressed early in the season while temperatures are low. Also the flowers of purple genotypes are white when they first appear and it may take several days before they turn purple. Because of this peculiar mode of expression, both white and purple flowers are observed on purple genotypes.

Isozyme Markers

Isozymes were first used in guayule by Zaiger, Soltis, and Brown (72, 73) who showed that Cal-3 diploid germplasm is highly heterozygous. Currently isozyme studies are being conducted at the University of California, Riverside, and at the USDA Water Conservation Laboratory, Phoenix. In a recent study, guayule plants with $2n = 36$, 54, and 72 chromosomes, as well as plants from AZ-101 and Gila-1, were compared for the banding patterns of 17 enzyme systems (74, 75). Seven of these enzymes (PGI, SKDH, IDH, MDH, GOT, TPI, and

EST) produced recognizable bands. Although direct genetic analyses of the above enzymes are not yet available, the observed banding patterns indicate that isozymes will provide useful genetic markers for guayule. An interesting observation was the identical banding patterns of Gila-1 and AZ-101 for all of the seven enzyme systems, which supports the view that those two germplasms are apomictic progenies of a single plant. Based on these results, as more isozyme markers become available in guayule, fingerprinting and variety identification may be performed with no difficulties.

Restriction Fragment Length Polymorphism Markers

The use of RFLP markers has become very popular in both new and established crops. Restriction fragment length polymorphisms are differences observed in the fragment length of restriction endonuclease-digested DNA. Genetically distinct individuals often display fragments of different sizes because the DNA between the sites where the enzyme cuts DNA strands may vary from individual to individual. RFLP markers possess several advantages over conventional genetic markers. Since, on average, a restriction enzyme will generate close to one million fragments by digestion of genomic DNA, and since there are over 100 restriction enzymes known to date, the potential number of those markers is virtually unlimited. In addition, since these markers are part of DNA itself, their interpretation is not affected by environmental influences, thus removing a major source of confusion.

Guayule genetics and plant breeding studies will benefit greatly from RFLP markers, especially if some of those markers could be associated with traits of interest such as rubber content and rubber quality. Preparations are under way to begin identification of RFLP markers in guayule. Progress in this area, however, will depend on the level of funding for future research.

ADVANCES OF THE PAST, POTENTIALS FOR IMPROVEMENT, AND DIRECTIONS FOR FUTURE RESEARCH

Among the new and alternative crops, guayule occupies a special place. Guayule commercialization will not introduce another food or fiber crop whose overproduction has already created difficulties for many farmers in this country. On the contrary, guayule would yield a strategic industrial product, that is at present entirely imported. It is ironic, however, that 100 years after the first large-scale rubber extraction in 1888 there is no commercial field of guayule anywhere in the world. Guayule has suffered from intermittent research efforts, and the limited advancements have been undermined by periods of neglect.

The first real advancement came in the late 1920s when Dr. McCallum succeeded in cultivating a wild plant for profit. Considerable advances were made during the ERP in the 1940s. Many talented scientists working on taxonomy, agronomy, physiology, plant pathology, processing, genetics, and breeding of the plant revealed a number of secrets about its reproductive biology, environmental influences on rubber biosynthesis, and other areas, as the list of references in every chapter of this book indicates.

In spite of a number of interruptions, progress has been made in all aspects of guayule research and development during the last decade. Following are some of the major achievements with respect to plant breeding and genetics research: development of higher rubber-yielding germplasms; identification of genotypes with regrowth ability for the development of cultivars suitable for multiple harvest; identification of genotypes with reduced and delayed postharvest rubber degradation; isolation of rubber particle proteins; identification of a morphological marker and a number of isozyme markers; and above all, establishment of sound plant breeding programs in California and Arizona with well defined short-term and long-term objectives.

New concepts and fresh approaches have emerged as researchers have become more familiar with their organism. Some of the concepts such as clipping or pollarding have been around for

a long time. In recent works, however, researchers are actively engaged in developing cultivars that could be harvested economically in two-year cycles. Also, the fact that most of the rubber is stored in the bark has been known for some time. The challenge of current genetics and plant breeding research is to maximize bark in future commercial cultivars. On the other hand, the new concept of processing only bark for high rubber quality is based on a recognition that bark rubber has considerably higher molecular weight. Whether or not processing only bark is economically feasible depends on the productivity of future varieties and the decortication costs.

Many areas of future research were highlighted in this chapter. As in the development of any new crop, particularly one being developed directly from a wild species, a great deal of time and effort is required. The selection and hybridization of apomictic plants, recurrent selection of the diploids, and backcrossing of the interspecific materials to guayule should continue. The following activities should be pursued: tissue culture and vegetative cuttings for the maintenance of the promising genetic stocks and improved cultivars; anther and pollen culture for production of homozygous genotypes; systematic screening for rubber yield components, especially higher bark/wood ratio; genetic analyses of apomixis; and a concerted search for useful genetic markers.

Molecular genetic research on rubber biosynthesis should continue, not because it will provide any immediate solutions to increase rubber yield or produce a commercial guayule crop as some people may expect, but because it will answer some fundamental questions regarding the molecular basis of rubber biosynthesis in guayule. In the long run this will be helpful to cultivar development. Judging from the slow and limited progress made in the area of genetic engineering of established crops, where many scientists are actively engaged in highly competitive research, it is unreasonable to expect extraordinary improvements in guayule by the genetic engineering approach. This by no means should discourage genetic engineering research in guayule, but rather should serve to encourage the allocation of basic research funds for research on the molecular biology of rubber biosynthesis.

Progress through plant breeding is scale dependent. A sustained, even an expanded and fully integrated, program of research for 10 to 15 years is needed to develop cultivars that will meet the challenges of commercial production.

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Chapter 5

Environmental Physiology and Biochemistry of Rubber Formation

Chan R. Benedict

TEMPERATURE

The regulation of rubber synthesis has been extensively studied in guayule (*Parthenium argentatum* Gray) (1-6). An outstanding feature of rubber synthesis in this plant is that it is stimulated by low night temperatures (1, 2). Exposure of plants to 27°C days followed by 7°C nights for four months induced a three- to four-fold increase in rubber compared to control plants grown at a constant 27°C day and night. The low night temperature specifically induced the synthesis of *cis*-polyisoprene and had no effect on the rate of synthesis of *trans*-isoprenoid resins. Van Staden (4) has demonstrated that rubber synthesis in guayule in South Africa is strictly a winter phenomenon. Downes and Tonnet (5) established that low-temperature phytotron conditions are more favorable than warmer environments for rubber content. It was suggested that since growth is not observed in the field below 11°C, winter conditions slightly higher than this temperature appear to be desirable for rubber synthesis.

Goss et al. (7) reported that the exposure of guayule plants to low night temperatures for six months stimulated the formation of rubber particles in the cortical parenchyma cells. In this study, the incorporation of [^{14}C]-acetate into polyisoprene in stem sections from plants exposed to low temperatures was several-fold higher than acetate incorporation into polyisoprene in

stem sections from control plants. This suggests that the enzymatic potential for synthesizing rubber is greater in stems from plants exposed to cold temperature. Bonner (3) has suggested that low night temperatures induce the expression of genes coding for enzymes involved in rubber synthesis. The mechanism of the cold induction of rubber synthesis has not been elucidated and it is the purpose of the following section to describe the effect of the low winter temperature of the fall and winter of the Chihuahuan Desert on rubber formation and on the rate of enzymatic incorporation of isopentenyl pyrophosphate (IPP) into rubber in field-grown guayule plants. The biochemistry of rubber formation in guayule is next addressed to demonstrate that the low temperature increase in the enzymatic incorporation of IPP into polyisoprene can in part be accounted for by an increase in polyprenyl (rubber) transferase activity.

PHYSIOLOGY

The data in Figure 1 show the progressive synthesis of biomass, resins, and rubber in field-grown plants from August 1985 to March 1986. The plants were transplanted to the field in the Chihuahuan Desert at Ft. Stockton, Texas, May 31, 1985. During an eight-month period from August to March, the biomass of the plants increased from 26.1 g/plant to 136.5 g/plant and the resins increased from 1.26 g/plant to 13.45 g/plant. The increases in biomass and resins were linear from August to December and the synthesis of both biomass and resins plateaued in late winter from January to March. In contrast, the amount of rubber in the guayule plants was low in August (18.0 mg/plant) and increased to 7,310 mg/plant in March. The amount of rubber formed in the plants paralleled the number of hours of temperature of 13°C and below to which the field plants were exposed during this period. These results show that the synthesis of rubber in guayule was stimulated by the low temperatures of the fall and winter of the Chihuahuan Desert.

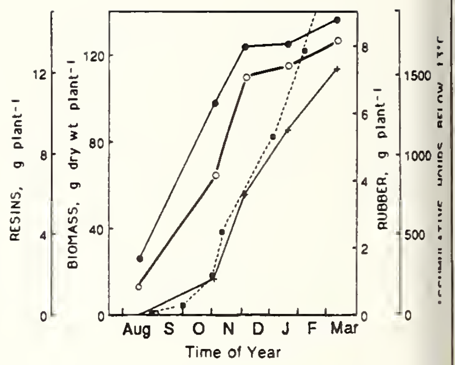

Figure 1. Synthesis of biomass (•—•), resins (o—o), and rubber (★—★) in field-grown guayule plants correlated to the accumulative hours below 13° C (■—■) at Ft. Stockton, Texas, from August 1985 to March 1986. Each data point for rubber, resins, and biomass represents the mean of five replicates.

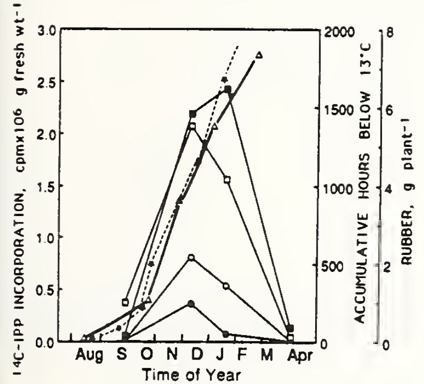

Figure 2. Rate of the enzymatic incorporation of IPP into cis*-polyisoprene in stem homogenates from the base of the stem (□–□) the base of the branch (■—■), the middle portion of the branch (o—o), and the meristematic regions (•—•) correlated to the accumulative hours below 13°C (★—★) and rubber deposition (Δ—Δ) from August 1985 to April 1986. Enzymatic rate and rubber deposition determinations were made from tissue samples from at least 10 branches of guayule plants.*

The data in Figure 2 show the rate of the enzymatic incorporation of IPP into *cis*-polyisoprene in stem homogenates prepared from the bark of guayule plants from the period of October through April of 1985-86. The enzymatic activity was measured in sections taken from mature stems from the meristematic region, internodes in the middle of the stem, and internodes at the base of the stem from plants in the same field plot as plants harvested for total biomass, resin, and rubber content. The enzymatic incorporation of IPP into rubber was low in all sections of the stem in October, increased in the sections in December and January, and declined in April. The decline in activity may be due to the coagulation of the rubber particles. The enzymatic activity in the stem homogenates was lowest in the meristematic region and highest in the internode sections from the middle part of the stem and from the internodes at the base of the stem. The increase in the enzymatic incorporation of IPP into rubber paralleled the increase in rubber formation in the guayule plant and the accumulated number of hours of 13°C and below. These data show that the enzymatic potential for rubber synthesis is increased in guayule plants exposed to low winter temperatures. However, to determine the specific effect of low temperature on the polymerization of IPP into polyisoprene by polyprenyl (rubber) transferase, the biochemistry of this enzyme reaction in guayule has to be established followed by measuring the enzyme activity under optimum assay conditions in plants exposed to cold temperature.

BIOCHEMISTRY

Most of the information on the biochemistry of rubber formation has resulted from studies on mevalonic acid (MVA) and IPP incorporation into *cis*-polyisoprene in *Hevea brasiliensis* (A. Juss.) Muell.-Arg. latex and in isolated washed rubber particles (WRP) (8-11). At the present time, there are two mechanisms to account for the in vitro incorporation of IPP into *cis*-polyisoprene: de novo synthesis of new rubber chains and the lengthening of existing rubber

chains (10, 11). Rubber particles isolated from *H. brasiliensis* contain a rubber transferase that catalyzes the polymerization of IPP into rubber. The polymerization reaction is stimulated by C_5, C_{10}, C_{15}, and C_{20} allylic pyrophosphate (allylic-PP) initiators and leads to the de novo synthesis of high-molecular-weight polyisoprene (10, 11). The finding that *trans* geranylgeranyl pyrophosphate (GGPP) can initiate rubber formation in vitro suggests that rubber molecules formed in vivo may possess a small number of *trans* double bonds at the isopropylidene end of the molecule. This agrees with the arrangement of isoprene units along the polymer chain of rubber isolated from *H. brasiliensis* as a dimethylallyl w-terminal unit, three *trans*-units, a long block of *cis*-units and a *cis*-a-isoprenyl terminal unit (12).

The incorporation of IPP into *cis*-polyisoprene in WRP by chain lengthening has been described as the transfer of a *cis*-1,4-polyprenyl group from *cis*-1,4-polyprenyl-PP catalyzed by rubber transferase (9). A soluble rubber transferase has been purified 350-fold from the serum fraction of the *H. brasiliensis* latex. The soluble rubber transferase is added to a preparation of WRP, Mg^{2+}, and IPP and catalyzes chain lengthening of existing rubber molecules on the surface of the rubber particle (8). The rubber transferase assay has been used in a number of laboratories to measure the activity of the enzyme catalyzing the incorporation of IPP into polyisoprene (13-15). However, chain elongation was not measured directly in the original assay of rubber transferase (9) and it is not clear whether the protein that stimulates the incorporation of IPP into polyisoprene in WRP does so by catalyzing chain elongation or by catalyzing the formation of allylic-PP initiators for the de novo synthesis of polyisoprene.

The biochemistry of the polymerization reaction catalyzed by polyprenyl (rubber) transferase has been studied in WRP isolated from stems of guayule plants (16). The data in Table 1 show the cofactor requirement for the incorporation of [^{14}C]-IPP into polyisoprene in a suspension of WRP isolated from crude extracts of guayule stems by gel filtration chromatography on an LKB Ultragel AcA34 column. The formation of [^{14}C]-polyisoprene is stimulated by Mg^{2+} and dimethylallyl pyrophosphate (DMAPP). The increase in the polymerization reaction with increasing concentrations of IPP is shown in Figure 3. The K_m for IPP calculated from the

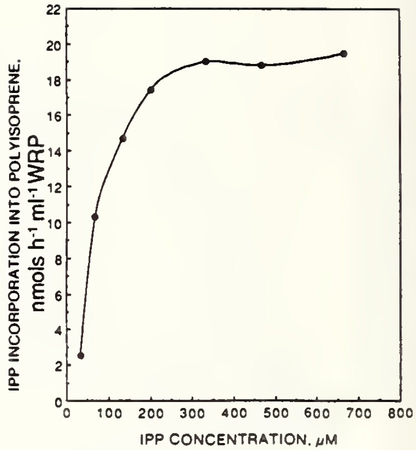

Figure 3. The effect of increasing concentrations of IPP on the rate of enzymatic incorporation of IPP into polyisoprene in WRP. The reaction mixture contained 0.15 μmol $MgCl_2$, 1.0 μmol GSH (pH 8.0), 50 nmol DMAPP, varying concentrations of IPP, and 100 μl WRP. The reaction mixtures were incubated at 25°C for 60 minutes.

Table 1. Cofactor requirements for the incorporation of [^{14}C]-IPP into polyisoprene in a suspension of washed rubber particles (WRP).

Reaction mixture[a]	[^{14}C]-IPP incorporation (nmol/hour ml WRP)
Complete	10.1
-GSH	6.0
$-Mg^{2+}$	2.6
Boiled	0.6
-DMAPP	0.8

[a]The reaction mixture contained 0.15 µmol $MgCl_2$, 1.0 µmol GSH (pH 8.0), 27 nmol [^{14}C]-IPP (2.29 KBq of radioactivity), 50 nmol DMAPP, and 100 µl WRP. The reaction mixtures were incubated for 60 minutes at 25°C.

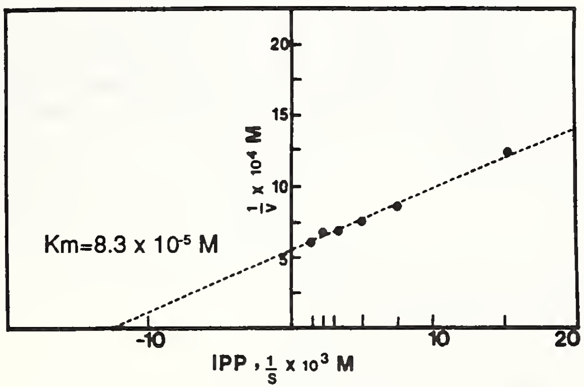

Figure 4. Double reciprocal plot of IPP concentration versus reaction velocity. The K_m for IPP for this reaction is 8.3×10^{-5} M.

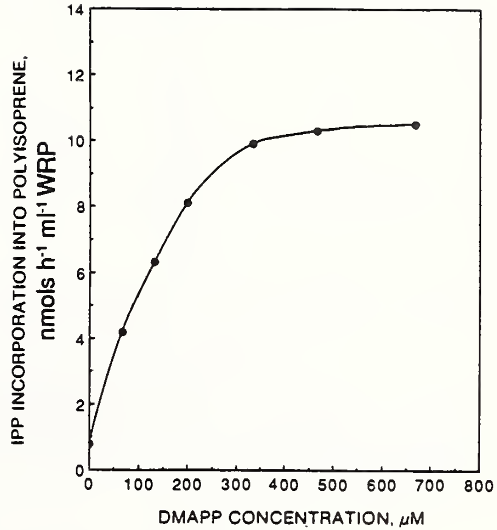

Figure 5. The rate of ^{14}C-IPP incorporation into polyisoprene with increasing concentrations of DMAPP (0-700 µM) in isolated rubber particles. The reaction mixture contained 0.15 µmol $MgCl_2$, 1.0 µmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (2.29 KBq of radioactivity), varying concentrations of DMAPP, and 100 µl WRP. The reaction mixtures were incubated at 25°C for 60 minutes.

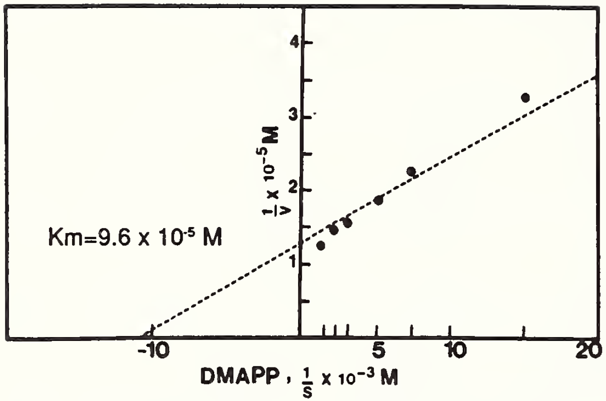

Figure 6. Double reciprocal plot of DMAPP concentration versus reaction velocity of IPP incorporation into polyisoprene in isolated rubber particles. The K_m for DMAPP for this reaction is 9.6×10^{-5} M.

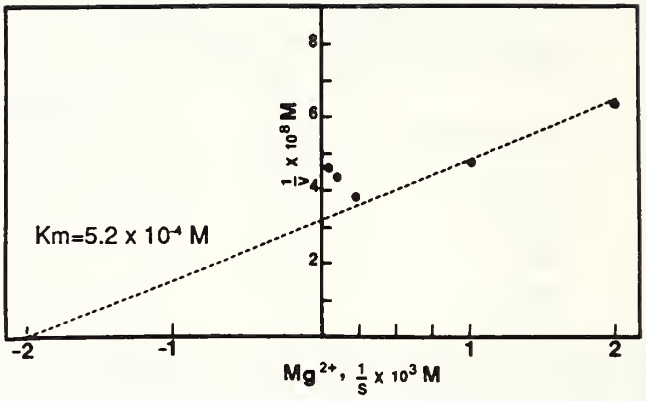

Figure 7. Double reciprocal plot of Mg^{2+} concentration versus reaction velocity of IPP incorporation into polyisoprene in isolated rubber particles. The K_m for Mg^{2+} for this reaction is 5.2×10^{-4} M. The reaction mixture contained 1.0 µmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (2.29 KBq of radioactivity), 50 nmol DMAPP, varying concentrations of Mg^{2+}, and 100µl WRP. The reaction mixtures were incubated at 25°C for 60 minutes.

double reciprocal plot of IPP versus reaction velocity (Figure 4) is 8.3×10^{-5} M. The increase in the polymerization reaction with increasing concentrations of DMAPP initiator is shown in Figure 5. The K_m for DMAPP is 9.6×10^{-5} M (Figure 6). The K_m for Mg^{2+} calculated from the double reciprocal plot of Mg^{2+} versus reaction velocity (Figure 7) is 5.2×10^{-4} M. The data in Figure 8 show that [^{14}C]-IPP is incorporated into polyisoprene over a three-hour period. The data in Figure 9 show the chromatography of the [^{14}C]-polyisoprene synthesized from [^{14}C]-IPP, Mg^{2+}, and DMAPP in the WRP on three linear columns of 1×10^6 Å to 500 Å of Ultrastyragel in

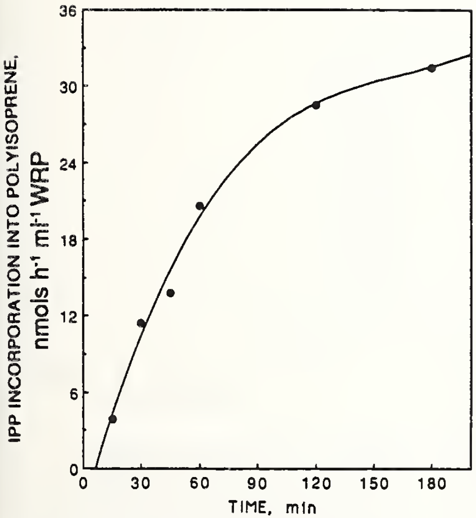

Figure 8. The effect of reaction time on the rate of IPP incorporation into polyisoprene in isolated rubber particles from guayule. The reaction mixture contained 0.15 µmol $MgCl_2$, 1.0 µmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (2.29 KBq of radioactivity), 50 nmol DMAPP, and 100 µl WRP. The reaction mixtures were incubated at 25°C.

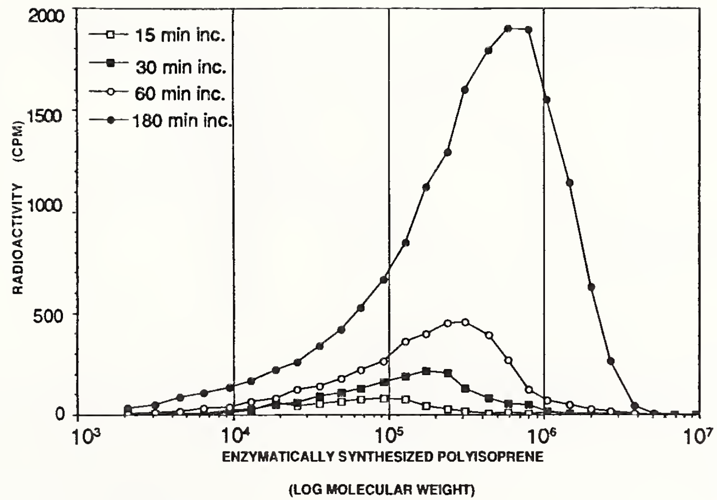

Figure 9. Gel permeation chromatographic analysis of the [^{14}C]-polyisoprene synthesized over varying periods of time (15, 30, 60, and 180 minutes) from [^{14}C]-IPP, Mg^{2+}, and DMAPP in the rubber particles isolated from guayule stem extracts. There is an increase in the peak molecular weight of the ^{14}C-polyisoprene from 70,000 in 15 minutes to over 750,000 in 180 minutes.

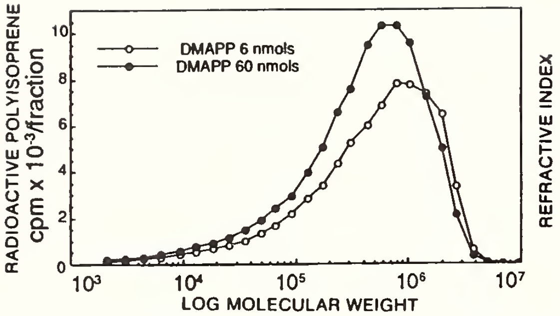

Figure 10. Molecular weight profile obtained from the gel permeation chromatographic analysis of the [^{14}C]-polyisoprene synthesized over a three-hour period with varying concentrations of DMAPP (6 and 60 nmol) in the reaction mixture. The reaction mixture in each of the 30 tubes contained 27 nmol [^{14}C]-IPP and 27 nmol Mg^{2+} in addition to DMAPP.

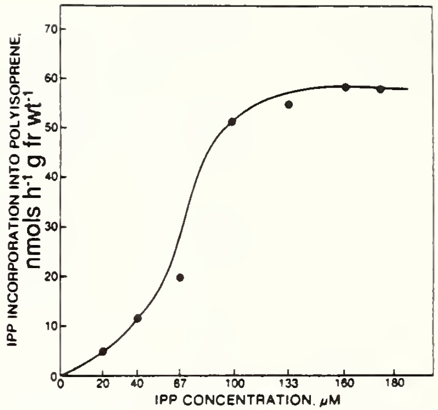

Figure 11. The rate of incorporation of [^{14}C]-polyisoprene into polyisoprene with increasing concentrations of IPP in crude extracts of guayule stems. The reaction mixture contained 0.15 μmol $MgCl_2$, 1.0 μmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (22.9 KBq of radioactivity), and 100 μl stem extract. The reaction mixtures were incubated at 25°C for 60 minutes. (Reprinted from Plant Physiology *with the permission of the Amerian Society of Plant Physiologists.)*

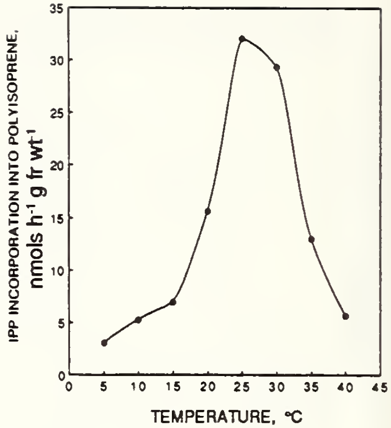

Figure 12. The effect of incubation temperature on the incorporation of [1-^{14}C]-IPP into cis-*polyisoprene in the stem homogenates of guayule. The reaction mixture contained 0.15 μmol $MgCl_2$, 1.0 μmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (22.9 KBq of radioactivity), 50 nmol DMAPP, and 100 μl WRP. The reaction mixtures were incubated at 25°C. The temperature optimum for the polymerase reaction is 25°C.*

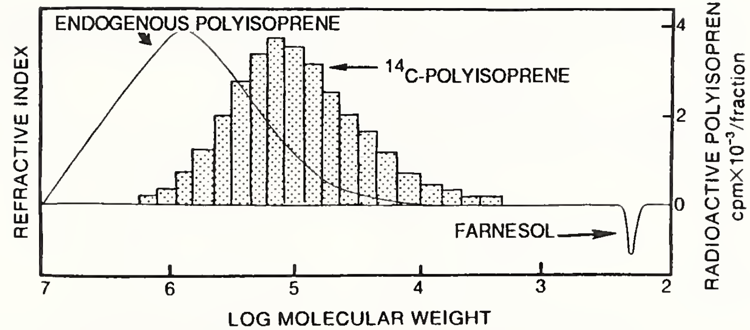

Figure 13. Gel permeation chromatographic analysis of [^{14}C]-polyisoprene enzymatically formed over a three-hour period from [^{14}C]-IPP in crude extracts of guayule stems. (Reprinted from Plant Physiology *with the permission of the American Society of Plant Physiologists.)*

a Waters Gel Permeation Chromatograph over a three-hour period. There is an increase in the peak molecular weight of the [^{14}C]-polyisoprene from 70,000 in 15 minutes to over 750,000 in three hours. From the data in Table 1 and Figure 9, it can be calculated that over 90 percent of the [^{14}C]-IPP incorporated into the polyisoprene is de novo synthesis. From the data in Figure 9 it can be seen that there is no incorporation of [^{14}C]-IPP into high-molecular-weight polyisoprene in short incubation times, which is indicative of chain lengthening of existing rubber molecules with IPP. The data in Figure 10 show the molecular weight profile of the rubber polymer formed with 6 and 60 nmol DMAPP in the reaction mixture. The [^{14}C]-rubber polymer formed at 6 nmol DMAPP compared to the polymer formed at 60 nmol DMAPP has a higher peak-molecular-weight and a higher percent of high-molecular-weight rubber chains. This is typical of most polymerization reactions under limiting concentrations of initiator and saturating concentrations of monomer.

Table 2. Incorporation of [1-^{14}C]-IPP into *cis*-polyisoprene in crude extracts[a] of guayule stems.

Reaction mixture[b]	[1-^{14}C]-IPP[c] incorporation (nmol/hour g/fresh weight)
Complete	73.5
-Mg^{2+}	32.9
-GSH	59.1
Boiled extract	1.5
-DMAPP[d]	61.9

Source: Reprinted from *Plant Physiology* with the permission of the American Society of Plant Physiologists.

[a]Extracts were made from plants sampled from field plots in December.
[b]The reaction mixture contained 0.15 µmol $MgCl_2$, 1.0 µmol GSH (pH 8.0), 27 nmol [1-^{14}C]-IPP (22.9 KBq of radioactivity), 50 nmol DMAPP, and 100 µl WRP. The reaction mixtures were incubated for 60 minutes at 25°C.
[c]IPP isomerase activity was 32.1 nmol 15 min^{-1} g fr wt^{-1}.
[d]20 to 100 nmol.

The cofactor requirement for incorporation of [^{14}C]-IPP into polyisoprene in crude extracts of guayule stems is shown in Table 2. The formation of radioactive polyisoprene is stimulated by the addition of Mg^{2+} and GSH. The formation of a greater amount of [^{14}C]-polyisoprene in the crude extracts compared to the WRP mixtures is due to more rubber particles in the crude extracts. The addition of 20 to 100 nmol of DMAPP to the crude reaction mixtures (Table 2) did not stimulate the incorporation of [^{14}C]-IPP into polyisoprene probably due to the presence of an active IPP isomerase furnishing saturating levels of DMAPP initiator. The formation of [^{14}C]-polyisoprene in the crude extracts with increasing concentrations of IPP is shown in Figure 11. The reaction velocity versus the substrate concentration displays a sigmoidal plot, which may indicate the presence of an allosteric polymerase or may indicate that the polymeri-

Table 3. The effect of cold temperature on polyprenyl transferase activity in crude homogenates from stems of field-grown guayule plants.

Harvest date	Polyprenyl transferase activity[a] (nmol/hour g/fresh weight)
October 28, 1986	12.1 + 1.9
November 19, 1986	37.9 + 7.4
December 9, 1986	86.6 + 15.1
January 6, 1987	144.3 + 17.5
January 26, 1987	42.5 + 5.8
April 7, 1987	4.2 + 1.1

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[a]Each data point is an average of determinations of three individual plants.

zation reaction is limited by the production of DMAPP from the IPP substrate at low concentrations of IPP. The polymerization reaction is saturated in the crude extracts at 133 µM of IPP. It was shown that the reaction was saturated at 1 mM Mg^{2+} and increased linearly with the addition of 0 to 100 µl of crude extract (16). The reaction was linear for 60 minutes at 25°C, 133 µM IPP, 1 mM Mg^{2+}, and 100 µl of crude extract. The data in Figure 12 show that the polymerization reaction has a temperature optimum of 25°C. Low temperatures do not stimulate the reaction. The chromatography of the enzymatically synthesized [^{14}C]-polyisoprene synthesized in the crude extracts on linear columns of 1 x 10^6 Å to 500 Å of Ultrastyragel is shown in Figure 13. The peak molecular weight of the [^{14}C]-rubber polymer is about 200,000 and the molecular weight distribution is 10^3 to 10^6. Over 90 to 95 percent of the radioactivity in the [^{14}C]-polyisoprene applied to the chromatographic columns was recovered in the [^{14}C]-

polyisoprene fractions. The profile of the radioactive polyisoprene was not coincident with the profile of the endogenous polyisoprene and shows that the average molecular weight of the enzymatically synthesized [^{14}C]-polyisoprene from the crude extracts is less than the molecular weight of the endogenous polyisoprene. These data also suggest that no nonspecifically bound [^{14}C]-contaminant was present, since it would be evenly distributed through the endogenous polyisoprene.

The data in Table 3 show the polyprenyl transferase activity in crude extracts of guayule stems sampled at different times during the fall and winter from field plots in 1986-87. Transferase activity was measured by determining [^{14}C]-IPP incorporated into polyisoprene at 133 μM IPP, 1 mM Mg^{2+}, and 100 μl crude extract for 60 minutes at 25°C. In plants exposed to the low fall and winter temperatures, the enzymatic formation of the [^{14}C]-polyisoprene by polyprenyl transferase activity in the crude extracts increases from 12.1 nmol/hour g/fresh weight in stems from plants sampled in October to 144.3 nmol/hour g/fresh weight in stems and plants sampled in January. The decrease in transferase activity in plants sampled January 26 and April 7 is probably due to the coagulation of the rubber particles and precipitation from the supernatant. The rate of the enzymatic synthesis of polyisoprene in crude extracts in October, November, December, and January is correlated with the accumulative number of hours of temperature of 13°C and below with an R^2 value of 0.989. The 12-fold increase in polyprenyl transferase activity in guayule plants exposed to low temperature agrees with Bonner's suggestion (3) that low temperature stimulates polyisoprene synthesis by inducing the expression of genes coding for rubber enzymes.

ACKNOWLEDGMENTS

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Chapter 6

Land Preparation

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Native stands of guayule persist within a wide range of climatic tolerances. Annual precipitation averages 25 to 35 cm and temperatures may range from -23° C to near 49° C (1). Distribution is generally at altitudes of 700 to 1,500 m in the Trans Pecos region of Texas, while some Mexican stands may occur at elevations of 1,800 to 2,000 m (2).

Although native stands are restricted to outwash fans and rocky slopes of calcareous soils, future cultivation will be limited to areas reserved for field-crop production. Site selection must include identification of suitable climatic and soil parameters. Soil is a dominant factor in guayule production because of its relation to moisture control and shrub growth (3).

SITE SELECTION

Guayule was established in coastal, central, and southern California, central Arizona, southeastern New Mexico, and west and southcentral Texas during 1942 to 1946 by the Emergency Rubber Project (ERP) (4). The ERP (5, 6) developed the following standards for surveying land to be converted to guayule production: physiography, soils, climate, water supply, biological factors, and economics of the community or individual farm.

Physiography. Mountains, hills, steeply undulating areas, eroded and gullied areas, swamps, lakes, and salt flats were eliminated by the exploratory survey of the region or state.

Soils. Potential soils for guayule cultivation were categorized based on profile and certain external characteristics. Class I was excellent for guayule production and encompassed fine and very fine sandy loams, loams, and silty loams with well-drained profiles. Slopes did not exceed 3 percent in irrigated fields or 7 percent in dryland, and injurious salts were absent. Loamy fine sands, clay loams, and silty clay loams were included in Class II. Claypans were not allowed and at least moderately adequate internal drainage was required. Class III, doubtful for guayule cultivation, covered the loamy sands and silty clays, which had moderate amounts of stones and gravel. Classes IV and V were not adequate for guayule production.

Climate. Climatic standards involved temperature and rainfall. Areas with minimum temperatures between -15° and -12° C were suitable for cultivation (4). Mean annual rainfall of 280 to 640 mm was declared suitable for dryland culture, but regions receiving less than 230 mm were rejected. Annual precipitation of 640 mm was set as the upper limit of suitability, 640 to 890 mm was considered doubtful, and over 890 mm unsuitable. Seasonal distribution of rainfall was also considered. Maximum rainfall in early spring and summer, with the minimum in late fall and throughout the cold season, was most desirable. Guayule plantings were predominantly located in low-rainfall areas. Experience by the ERP led to later modifications of the recommended requirements. Minimum temperatures were set at -9° C, unless winters were dry and the shrubs dormant for an extended period prior to the minimum. The original lower level of precipitation for dryland production was raised to 380 mm except in regions with cool, foggy summers.

Water supply. Water supply was judged according to quantity and quality. The rate of flow requirements were greater in arid regions, and allowable salt content varied with soil depth and permeability. Water requirements for land classes I through IV were proposed for arid, intermediate, and coastal areas (6). Water amounts recommended for Class I were 1,500, 1,250, and 850 m^3/ha, respectively, for arid, intermediate, and coastal sites. Based on two years of irrigation experience, the original standards were reduced by one-third.

Biological factors. Hazards of biological factors such as insects, disease, weeds, and natural

vegetation were considered before land selection. The accepted methods, standards, and remedial measures pertinent to common farm crops were applied to guayule. Several insects causing damage to guayule were known: wire worms, darkling ground beetles, grasshoppers, carrot beetles, lacebugs, stem borers, termites, lygus, and ants. Crown rot, root rot, and wilt were sometimes damaging to guayule; but infested areas were not delineated in the early surveys. Later soil and tract reconnaissance surveys in Texas designated fields infected with cotton root rot. Plant losses were minimized on well-drained, light-textured soils by proper irrigation practices. Nematode infestations were considered in the selection of nursery sites and experimental fields. Tracts with heavy infestations of morningglory, bermudagrass, or johnsongrass were avoided. Ecological surveys were based on plant composition of the various vegetative associations. These studies were more common in Texas where suitable rangeland sites were available.

Economic factors. Economic elements considered were farm size, tenancy, land values and rentals, farm labor requirements, types of crops, and effect on the community of introducing guayule as a new crop.

The above surveys dealt primarily with methodology and definite physical and environmental factors; nonetheless, other considerations were also important. Mill-unit areas, comprising 4,510 ha or one mill unit, were emphasized. Transportation costs were diminished by closely located production sites. Speculative land was prominent, with many producers desiring to get land developed at government expense. Finally, the relative value of suitable land for various uses was considered. Because natural rubber production was important, the guayule program was concentrated in one of the United States' most intensive food production regions.

MECHANICAL PREPARATION

Requirements by the ERP for soil preparation for guayule did not differ significantly from those for many other farm crops. The soil had to be loosened to a depth at least equal to that of planting, large amounts of crop debris could not be present, and the soil needed to be well-pulverized and compact (5). Land for planting in 1942 and 1943 became available late in the growing season; therefore, there was insufficient time to handle any crop residue. Lacking the opportunity for soil incorporation, residue was removed by raking and burning.

Changes were made in land preparation techniques as the ERP progressed. Initial preparation involved loosening the soil to allow penetration of the planting shoes. Chiseling crop debris was substituted for plowing. Experience during the 1942 and 1943 planting seasons indicated that the influence of organic matter on soilborne diseases was not serious; consequently, crop residues were incorporated into the soil during the 1944 season. Common crop residues were cotton stalks, alfalfa, barley straw, tomato stalks, and melon vines. The number of mechanical operations required for field preparation was reduced by avoiding plowing or chiseling when the soil was either too wet or too dry, preventing the formation of large clods by disking or harrowing following plowing and chiseling, and minimizing floating or dragging.

The majority of the ERP plantings were on cropland cultivated the previous growing season. During 1978-1981, Bridgestone/Firestone (formerly the Firestone Tire and Rubber Company) established about 80 ha of guayule near Fort Stockton, Pecos County, Texas. A dense stand of mesquite (*Prosopis glandulosa*), creosotebush (*Larrea divaricata*), and tarbush (*Flourensia cenura*) inhabited the site that had been farmed for a short period during the 1950s. Following root plowing 60 to 75 cm deep, the brush was raked, stacked, and burned. Rocks were removed mechanically and by hand before double disking followed by floating. Sites with deeper soils were plowed 50 to 60 cm deep before disking.

SEED TECHNOLOGY

The guayule inflorescence, a compound, one-sided cyme, terminates the growth of the primary shoot and subsequent branches (2). Flowers are borne in heads on a common receptacle. Each head contains five fertile ray-florets, each with two attached subjacent sterile disk-florets. The mature seed, or achene, is enclosed by two sterile disk-florets, and a subtending bract, together with a persistent ligule. As the seed matures, the flower head disintegrates. That is, the ray flowers that bear the seed remain attached to their adjacent disk flowers and bracts, and fall away as a whole (7). The remaining disk flowers also drop off, leaving behind the five bracts attached to the receptacle.

Pollination

Guayule is both wind- and insect-pollinated. Viable guayule pollen can be carried as far as 775 m by wind (8), and the morphology of the outer surface of pollen grains also suggests an adaptation to insect pollination. Gardner (8) found that ladybird beetles, lygus bugs, and cucumber beetles were effective pollen carriers. Germination of seed from open pollinated plants and plants caged with insects was greater than for plants caged without insects. Mamood, Ray, and Waller (9) reported similar results from plots caged with honey bees. The increase in germination rates may be explained by the necessity of pollen for endosperm development and the attainment of normal embryo size (9, 10).

Field Production

Native stands of guayule normally flower in the summer; however, flowering may occur at any time depending upon available rainfall (2). Under irrigation, plants flower almost continuously

during the growing season. Irrigated plantings in the Bakersfield District of the ERP produced seed throughout the growing season, and four to six collections were made. Shrubs in dryland fields grew slowly and the main seed crop of the first growing season matured in early September, late June, or early July in subsequent years (11).

The percentage of guayule seeds filled or viable fluctuated tremendously during ERP operations (5). Extremes of 0 to 70 percent were recorded, but 10 to 45 percent was normal. This variability was attributed to several factors: a) embryos created without fertilization taking place, b) lygus bugs feeding on flowers, c) high temperatures, and d) dormancy. Dryland plantings produced better-quality seed than irrigated sites, and the quality of seed collected in nursery plantings was usually too low for general use. Studies in Arizona have shown that percentage seed germination and weight of viable seed harvested decreased with declining water levels (12). Seeds from two guayule selections, N576 and N593, were hand harvested according to water treatment one and two years after planting. Ray, Coates, and Livingston (13) demonstrated that hedging promoted production.

Lack of flowering and subsequent achene development may lead to the use of assimilate in other plant functions (14). Four guayule lines (N396, N593, 11591, 11619) were tested in Arizona under three flowering regimes to determine the influence of flowering upon rubber production. Significantly greater rubber yields were measured when shrub inflorescences were clipped just before dehiscence. The increase may be sufficient stimulus to reduce or eliminate flowering in certain commercial operations.

Harvesting

Guayule seed shatters readily when ripe and can be removed from the plant easily by hand or by mechanical means. The ERP used both mechanical- and hand-collection methods depending upon existing conditions.

Mechanical. The Intercontinental Rubber Company (IRC) developed a vacuum harvester, which proved to be inefficient. Machines constructed during the ERP featured rotary brushes that forced the seeds into collection pans (5). The machine could cover 5 to 8 ha/day, collecting from 120 to 600 kg of uncleaned seed. During 1943 over 500,000 kg (uncleaned, unthreshed field weight) of seed were harvested at an average cost of $0.22/kg as compared to $0.66/kg for hand harvesting in 1942. Yields based on machine collections did not reflect the total produced. Wind, disturbance by hoeing crews, and cultivation and furrowing dislodged up to one-half of the ripe seed. Harvesting machinery removed 33 to 66 percent of mature seed remaining on the shrubs. Harvested seed represented, on the average, 25 to 30 percent of the total seed produced.

A nondestructive guayule seed harvester was developed by the University of Arizona (15). The Guayule Administrative Management Committee Seed Increase Program constructed harvesters using cotton pickers as power units equipped with headers that could be adjusted to variable heights. Subsequently, a new harvester was developed by the University of Arizona utilizing a John Deere 9900 cotton picker with a hydrostatic transmission. The machine was equipped with a ventura air system that eliminated seed passage through the fans and subsequent damage. Further modifications were made with the headers and a ground seed-harvesting head was added (16, 17).

A gasoline-powered backpack vacuum insect net has proven to be a satisfactory mechanical harvester for small areas (18). An average of 135 achene complexes, or 94 percent of the total available, was harvested in a greenhouse test. Mobility allows mechanical harvesting of native populations and cultivated plots.

A harvester consisting of an industrial vacuum cleaner on a 55-gallon drum, and powered by an electric generator, was constructed by Bridgestone/Firestone (19). The equipment was mounted on a high-profile tractor. Results indicated that a satisfactory two-row tractor-mounted harvester could be fabricated with the equipment.

Hand collection. Hand harvesting was common during the early development of the ERP, especially during 1942, when there was an urgent need for all available seed. Collections averaged about 9 kg of uncleaned seed per day per worker (5). Hand harvesting was practical when machines were unavailable, seed quantities were scarce, or maximum yields were necessary.

Timing. Frequent field inspection during the ERP indicated the proper time for seed harvesting. Seed heads were tan to brown in color and shattered easily when ripe. Irrigation and cultivation were also correlated with harvesting. Several factors influenced the frequency of seed collection from a given tract including cultural operations, irrigation, uniformity of ripening, and winds. Two or three harvests were conducted on dryland sites and as many as five on irrigated areas.

Cleaning and Handling

Initial cleaning involves removal of coarse trash such as stems, leaves, soil or sand, clusters of sterile florets, insects, and weed seeds. Floral parts enclosing seed, leaves, and other particles that are of similar weight make cleaning difficult. Prior to 1942, neither the IRC or local seed houses had perfected a suitable seed cleaning procedure. The ERP removed coarse trash collected with the seed by feeding the harvested material over a power-driven shaker or scalping screen (11). Seed was then processed through a small clipper cleaner consisting of three vibrating screens: a) a top screen of Number 10 round holes for removing oversized material, b) a middle screen of 13 x 13 mm slots for separating the clusters of sterile florets from the seed, and c) a bottom screen of Number 7 round holes for eliminating fine trash. Different screen sizes could be used depending on the seed size in various lots. The cleaning process was supplemented by a gravity separator that helped to separate filled seeds from empty seeds. During 1942 the ERP collected 81,720 kg of clean unthreshed seed, and 161,000

kg in 1943 (5). Seed threshing was developed late in the ERP when a trial with the Prater Hammermill indicated that the floral parts could be removed from the achenes with little damage to the seed (5). The Forsberg Burr Clover Huller was adopted because of its high capacity effectiveness, and minimum seed injury. Gravity separation was more effective with threshed seed and 180 kg/hr could be processed. Subsequent studies revealed that threshing was beneficial and actually improved seed viability. Unthreshed seed required special chemical treatment to overcome dormancy and achieve short-term germination.

A double-belt thresher has been evaluated for threshing large volumes of guayule seed (20). It consists of two 0.305-mm-wide rough-surfaced conveyor belts operating at 0.00 to 1.55 mm clearance. Threshing effectiveness and capacity depend on belt speed ratio, belt texture, and feed rate.

The University of Arizona process involved removing large debris and leaves by a Seedburo Model 36 Super Seed Scalper before threshing in either a Ferrell-Ross Scarifier or Forsberg Red Clover Huller (21). Threshed material was cleaned initially by a Ferrell-Ross Clipper M-2B Fanning Mill equipped with 2.0 x 1.2 mm sieves, followed by a second operation with a 4 x 22 mesh screen and 1.2 mm sieve or in a Clipper Office Tester. Seed was then placed on an Oliver Model 30 Gravity Separator.

Tipton, Carver, and Blackwell (18) developed an achene cleaning technique adapted from the ERP procedure. This technique consisted of three stages: a) preliminary cleaning with the Clipper Cleaner to remove stem and leaf fragments, and disk flower clusters, b) threshing with a Burr Clover Huller to separate achenes from the enclosing floral parts, and c) final cleaning. The final cleaning step involved manual screening followed by separation in a continuous-forced-air seed blower to remove fine trash and empty achenes.

The Los Angeles State and County Arboretum (LASCA) also modified the original ERP cleaning procedures (22). Harvested material was threshed in a Forsberg Number 2 Huller equipped with rubber cylinder and liner. Leaves, inflorescence stalks, and other extraneous

material were removed from the collected material with a Number 16 round-perforated metal hand-testing screen prior to the threshing step. Further separation of seed and floral parts was accomplished with the Clipper Cleaner or Clipper Office Tester. Three sieve sizes were used: a) 2 x 6 mm (oblong), b) 1 x 5 mm (oblong), and c) 2 x 2 mm (square). The sieves were paired in use, with the 1 x 5 mm (oblong) being continually used. Seed separation required two steps: a) the clipper shoe was fitted with the 2 x 6 mm sieve on top and the 1 x 5 mm sieve on the bottom (only seed collected in the left and bottom receptacles was saved), and b) the 1 x 5 mm sieve sat on top with a 2 x 2 mm sieve on the bottom (seed dispensed into the bottom receptacle was saved). Seed from the clipper was separated further with an Oliver Model 30 Gravity Separator.

A modification of the LASCA procedure was developed by Bridgestone/Firestone (19). Preliminary cleaning of harvested material included passing the unthreshed seed through a Number 16 round-hole hand-testing screen before threshing in the Forsberg Model 2 Huller/Scarifier. A third step was added to the Clipper Cleaner process: only a 1 x 1 mm sieve (square) was placed on the bottom, and seed dispensed into the bottom and middle compartments was retained. Seed from the left receptacle was further separated in the South Dakota Seed Blower, and finally passed through a 1-mm round-hole hand-testing screen. All seed obtained was placed in pentane for one to two minutes and floating seed was discarded.

Cleaning procedures at The Texas Agricultural Experiment Station Guayule Research Site include passing the unthreshed seed through a 6 x 6 mm hail screen and a Number 12 round-hole hand-testing screen prior to threshing in the Forsberg Model 2 Huller/Scarifier (23). A fourth step was added to the Clipper Cleaner process of Firestone's: a 2 x 2 mm sieve (square) is placed in the top position and a 1 x 1 mm sieve (square) on the bottom. After separation in the South Dakota Seed Blower, the seed was comparable to lots commercially cleaned.

Seed Storage

Storage at 4 percent moisture in airtight drums was adopted by the ERP for long-term storage (5). Seed to be used within two to three years was stored, allowing for aeration and humidity. Thus a greater percentage of the seeds lost their dormancy, which lessened the need for chemical treatment to ensure germination. Storage at low temperatures was not found to be essential for guayule. Benedict and Robinson (24) recommended that at temperatures of 20° to 25° C, guayule seed should be stored at a relative humidity of about 30 percent. Seed stored at humidities over 50 percent declined in viability. Chandra and Bucks (25) suggested that cleaned, conditioned seed should be stored at 10° C.

Germination and Dormancy

Germination of freshly harvested guayule seed is exceedingly low. This delayed germination is caused by two dormancy factors: a) an inner seed coat dormancy, which may last 12 months or longer, and b) an embryo dormancy of about two months duration (24, 26, 27). The inner seed coat is impermeable to gas exchange, but can be oxidized by chemical treatment. McCallum (28) was able to reverse delayed germination partially by treating seeds with a solution of calcium or sodium hypochlorite containing 1.5 percent available chlorine. Benedict and Robinson (25) demonstrated that in addition to sodium hypochlorite, other chemicals (hydrogen peroxide, perchloric acid, nitric acid) also broke seed dormancy. Conversely, Federer (29) indicated that sodium hypochlorite actually retarded seedling emergence. Emparan and Tysdal (26) reported that exposure to light and treatment with 0.75 percent sodium hypochlorite completely broke seed dormancy. Germination of freshly harvested, treated achenes was equal

to that of seeds stored 84 days. Hammond (27) concluded the following: a) that embryo and inner seed coat dormancy of freshly harvested achenes were completely broken by continuous exposure to daylight, b) that gibberellin substituted for light in breaking both dormancies, but sodium hypochlorite was equally effective only when supplemented with light, and c) that germination was increased by reducing the depth of sand covers of untreated and sodium-hypochlorite-treated seeds because of light transmission. Naqvi and Hanson (30) determined that the optimum concentration of NaOCl required for breaking seed dormancy was higher for younger than older seeds. Seeds were treated with equal parts of gibberellic acid (200 ppm) and NaOCl (1.0 percent for fresh seed and 0.25 percent for one-year-old seed). Large seeds emerged better at all depths to 18 mm after treatment versus medium or small seeds. Guayule chaff, floral parts enclosing the achene, may influence seed dormancy (31). Experiments have shown that aqueous extracts of guayule chaff and seed coat inhibited the germination and radicle growth of guayule, lettuce, and tomato.

Recent advances in seed treatment techniques have shown that conditioning enhances germination and the development of normal seedlings over a broad temperature range. The conditioning process involves imbibing seeds under aerobic conditions in a medium containing: a) 25 percent polyethylene glycol (MW8000), b) 10^{-4} mol gibberellic acid, c), 0.05 percent potassium nitrate, and d) 0.1 percent Thiram adjusted to pH 8.0 with a saturated solution of calcium hydroxide (25, 32, 33). Seeds are treated at 25° C in continuous light for three to four days. After conditioning, the seeds are air dried on blotters for two to three days at room temperature and stored in airtight containers at 10° C. The conditioning medium is designed to break dormancy and promote germination under acute osmotic stress.

Guayule seedlings are particularly sensitive to damping-off diseases caused by species of *Phythium*, *Rhizoctonia*, *Fusarium*, and other fungi. Thiram at concentrations below 10 µg/seed has been effective in controlling fungi in the seed-conditioning procedure and does not affect seed viability or vigor (32, 34).

PLANTING

Cultivated guayule stands have been established by: a) transplanting nursery-grown seedlings, b) transplanting seedlings propagated in the greenhouse, and c) direct seeding in the field. Transplanting nursery stock was used exclusively by the IRC and ERP. More recently, Bridgestone/Firestone transplanted about 80 ha of guayule with greenhouse seedlings, and the Gila River Indian Community established over 160 ha. Direct seeding was used experimentally during the ERP and is now the focus of several research programs throughout the Southwest.

Transplanting: Nursery Stock

Seedling production techniques developed by the IRC and adopted by the ERP consisted of the following: a) selecting land with good soil and level or gently sloping terrain, b) installing underground water mains feeding overhead sprinklers, c) sowing thickly with mechanical drills on beds, and d) topping, undercutting, lifting, and transplanting in the field after one growing season in the nursery (5, 11, 35-37).

Nursery propagation and cultural practices. The ERP nursery procedure involved dividing each 15-m space between irrigation pipelines into 9 or 10 beds, 1.2 x 122 m long, and separated by narrow paths for the wheels of the cultivator (11). Bandsowing machinery developed by the IRC was used because competition within the 5-cm-wide bands resulted in a compact form desired for transplanting. Optimum sowing rates involved planting enough seed for the establishment of 250 to 300 seedlings/m^2 of bed surface at the time of transplanting. Sand and native soil were used as coverings, depending on the type of soil in the nursery. Depth of cover varied from 0.25 cm for fine sands to 0.48 cm for coarser material. Sand covering allowed for better seedling emergence versus soil covering. Night temperatures, in

part, dictated the nursery planting season. Germination and emergence declined progressively when night temperatures fell below 16° C (11). The growing season should be long enough to produce seedlings of a usable size. Preferred sowing seasons in California were: Salinas—May 15 to July 15, Carlsbad—February to December, and San Clemente—March through October.

During germination and emergence, the surface of the beds was kept continually moist. After the plants had developed a substantial root system, irrigation frequency was decreased and the amount of water applied increased (11).

Cultivation was delayed until the seedlings were well established. Dense weed infestation sometimes required cultivation, and strict care was taken not to disturb the seeded beds (11, 38). Following seedling establishment, initial cultivations were shallow and later in the season the tracts were chiseled 10 to 15 cm deep. Mechanical thinning of directly seeded stands was attempted during the ERP. Marginal success was attained by cultivating (with sweeps) perpendicular to the seeded rows. Hand thinning, used by vegetable growers of the area, was adopted. Thinning and weed control could be accomplished in one operation. Top pruning, limited to spring and early summer plantings, opened the plant canopy and promoted drying of the soil surface. Pruning limited disease problems and stimulated plant growth. Clipping occurred when the seedlings were 8 to 10 weeks old.

Damping-off was one of the first diseases to become apparent in the early stages of seedling development (39-43). Additional disease problems facing guayule included *Verticillium* wilt, crown rot, *Sclerotinia* rot, *Botrytis* rot, leaf spot, and root knot. However, by sowing on soils having good drainage, using good tillage practices, and reducing irrigation once plants were established, disease problems were not serious (38).

Where soil fertility was adequate for normal growth, addition of fertilizer had little effect on guayule (44). Studies revealed that boron, copper, iron, potassium, magnesium, manganese, phosphorus, and zinc had no significant effect on growth in nurseries on good soils. Guayule responded to nitrogen and phosphorus; however, the effects were not evident until competition

among plants occurred. Fertilizer was applied before planting to low-fertility soils allowing for even distribution and immediate availability for plant use. Delayed applications were made on soils of average fertility. Late growing-season applications were too late for plant uptake, and produced succulent growth when shrubs were approaching dormancy.

Modern herbicides were not available during the ERP; therefore, oil sprays and hand weeding were employed for weed control. Stove oil was applied in a 1:3 combination with water; 27 l of solution was applied to each bed when weeds were in the cotyledon stage. Successive applications were sometimes necessary (5). Several chemicals were screened for weed control but most were injurious to the guayule (44). Sodium arsenite completely killed nursery stock, while zinc sulfate and borax retarded growth. Aluminum sulfate had little effect on guayule and was marginally toxic to weeds. Kerosene was not particularly harmful to guayule and gave good weed control. Whitworth (45), during the early 1950s successfully controlled weeds with Varsol at 280 to 350 l/ha when weeds were in the cotyledon stage.

A limited degree of insect injury was experienced in the California nurseries. Grasshoppers were the most destructive throughout the Southwest, and were successfully controlled by poison bait recommended by the Bureau of Entomology and Plant Quarantine. *Lygus hesperus* occurred throughout guayule plantings in California. The insects reduced the weight and viability of seed and sometimes fed on growing stem tips causing local cessation of meristematic activity and growth (46, 47). Arsenical and DDT dusts were used for control. Rodents and birds sometimes fed on nursery seedlings and seeds. Rodents were effectively controlled with systematic hunting, trapping, and poison baits (38). Birds fed on seed immediately after sowing. Frightening by shooting or windblown noisemakers was effective for control.

Nursery seedlings were hardened, or gradually brought to dormancy, by slowly reducing irrigation water (5, 11). The hardening process began when the plants reached a suitable size for transplanting, preferably at least 30 days prior to lifting. Hardening prepared the plants for topping, root pruning, lifting, and transplanting. Shrubs grown under high moisture stress, as

compared to lightly stressed plants, were able to resume growth quickly after transplanting and withstand adverse growing conditions with a higher survival rate (48).

Preparation of nursery seedlings for transplanting involved digging or lifting, and pulling and packing. Prior to digging, the plants were clipped or pruned about 4 cm above the soil surface. Erickson and Smith (49) recommended pruning at 2.5 cm or removing seven-eighths of the top within three days of digging. Guayule could be successfully transplanted under irrigation throughout the year. Reduction in transpiration was the primary physiological benefit of clipping (50). Digging involved undercutting the seedlings with a sharp blade fitted with lifting fingers. Pulling crews followed, removing the shrubs from the soil and packing them in crates for transport to transplanting sites or storage. Properly packed seedlings could be stored for 30 days at 0° to 33° C at a dry weight moisture content of 50 to 55 percent (5).

Mechanical transplanting. The ERP began transplanting nursery stock in 1942 with four transplanting machines fabricated by the IRC. A standard four-row Holland machine was subsequently adopted by the ERP, and approximately 100,000 transplants could be set out per nine-hour-day. Planting was done on tilled beds at least 20 cm deep. Plant spacing was generally 71 x 61 cm on irrigated land (23,000 plants/ha), and either 71 x 61 cm or 71 x 51 cm on dryland sites (5, 11). The planting season extended from about December 1 to March 15 or 20. Transplanting was completed early enough in the spring to capitalize on at least one substantial rainfall before the dry season.

Transplanting: greenhouse seedlings. Bare root transplanting has been largely replaced by the use of greenhouse seedlings 4 to 10 weeks old. Naqvi and Hanson (51), Fangmeier et al. (52), and Gonzalez and Rektorik (53) have described greenhouse equipment and operations.

Greenhouse operations. Initial greenhouse operations involved planting seeds in styrofoam or plastic seedling trays, peatmoss pots, plastic pots, peat pellets, and other containers filled with growing media. Seedlings grew best when greenhouse temperatures ranged from 21° to 32° C. Watering was critical since the young seedlings were sensitive to minor changes in

moisture of the media. Several waterings per day may be required. Tipton (54) concluded that once seedlings emerged, irrigation frequency could be reduced. Optimum growth required the media to be kept moist since large plants in small containers wilted rapidly under deficient water conditions. Fertilization usually included the following: a) constant and/or scheduled application of water-soluble fertilizer mixed with the irrigation water, or b) slow-release compounds incorporated in the growing media. Lime was sometimes added to the rooting media or irrigation water. Fungi associated with damping-off (*Pythium*, *Rizoctonia*, *Fusarium*, and *Phytophthora*) were controlled with a combination of Truban and Terrachlor (51). Insects such as aphids, loopers, mealybugs, whiteflies, thrips, and mites were common greenhouse pests. Naqvi and Hanson (51) obtained effective control with the following chemical sprays: Orthene, Metasystox R, Comite, and Dipel. Rotation of chemicals was important to avoid the evolution of resistant strains. Stone (55, 56) also evaluated several insecticides for control of problem insects in the greenhouse. Seedlings were hardened before transplanting by moving them outside, removing the top of the greenhouse, or trimming to a 60-mm height when about six weeks old (52).

Mechanical transplanting. Transplanting has employed numerous types of machines. The most common transplanters have positive-action fingers that place the seedlings at a specified depth. Transplants must be manually placed in the fingers with some models; however, semiautomatic or automatic units are also in use.

Cultural Practices: Field Transplants

Cultural practices for transplanted field stands include irrigation, cultivation, fertilization, pest control, and shrub conditioning. Many practices can be related to common procedures used in other row crops grown in the area.

Irrigation. Experience during the ERP proved that guayule could be produced in areas

where irrigation water was insufficient for cultivation of most vegetable crops, alfalfa, and cotton. Irrigation methods were similar to those used in the production of other closely spaced row crops (5). Furrow irrigation was primarily used in California; however, some border and flood irrigation was successful in Texas and Arizona.

Sprinkler irrigation was not used extensively during the ERP because of the lack of equipment, and most fields were already under furrow irrigation. However, limited tests indicated that sprinkler irrigation would be satisfactory for guayule production. Hunter and Kelley (57) reported that the greatest shrub and rubber yields on sandy loam soils occurred under high levels of moisture; conversely, on silty clay loams, the highest yields were produced on sites receiving the lowest moisture levels. The integrated value of the moisture tension function was lowest for the sandy soil, and after absorbing the water held at low tension, the plants were immediately under high moisture stress. Kelley, Hunter, and Hobbs (48) and Benedict, McCrary, and Slattery (58) found that nursery seedlings grown under high moisture stress had higher rubber content as compared to plants under low moisture stress. Results indicated that rubber accumulations could be forced by alternating periods of low and high moisture stress (5). High-stress periods must not be too short and, to induce maximum yields, should not occur during the winter. Tingey and Clifford (59) discovered that rubber production in 18-month-old transplants under moderate irrigation averaged 306 kg/ha, and 250 and 254 kg/ha, respectively, under light irrigation and no irrigation. Retzer and Mogen (60) learned that greater rubber percentages occurred as a result of frequent but moderate periods of moisture stress. The ideal situation would include producing two-year-old shrubs with an average rubber content of 7 to 9 percent, and biomass of 1,800 to 3,100 kg/ha with the application of 76 to 127 cm of water.

Irrigation frequency under furrow irrigation may vary from three to seven days depending on bed, soil, and water conditions (61). Miyamoto, Piela, and Gobran (62) applied 2 cm of water weekly for five weeks and obtained over 95 percent survival in the spring. Survival was only 80 percent during summer planting. Bucks, Nakayama, and French (63) transplanted in the

spring near Mesa, Arizona, and recorded 95 percent or better survival. Plots were furrow irrigated with 11 cm of water immediately after transplanting, and sprinkler irrigated once or twice a week for seven weeks with 1.8 cm per application. Transplants can tolerate and survive under saline water, but growth rates are retarded (61). Survival was 95 percent or greater in spring transplants furrow irrigated with 4.6 dS/m water. Summer planting when maximum daily temperatures reached 37° C resulted in reduced survival. Crown volume decreased 25 and 50 percent when water salinity increased from 0.8 to 4.6 dS/m, respectively.

Cultivation. Cultivation of field plantations during the ERP incorporated the use of small agricultural-type tractors fitted with four-row, integrally mounted row cultivators. This equipment was used for the maintenance of other row crops in the area; the same equipment can be used for agronomic practices today.

Fertilization. Bonner (64) concluded that guayule growth and rubber yields were: a) affected by nitrogen more than any other element, b) diminished in phosphate-deficient plants, c) independent of calcium and potassium concentrations, and d) depressed under conditions of low magnesium. Tingey and Foote (65) studied the effects of fall planting spacing, irrigation, and fertilization on rubber production in one-year-old guayule transplanted in December 1942. Applications of 118 kg/ha ammonium phosphate, 15 kg/ha nitrogen and 30 kg/ha phosphoric acid, occurred in July and November 1943, respectively. Shrubs irrigated and fertilized in the fall produced the greatest rubber content, which was progressively higher at closer spacings. Rubis (66) applied four fertilizer treatments in a guayule-seed-increase plot near Marana, Arizona. The compounds were applied at 112 kg/ha of actual nitrogen and included: Urea (46-0-0), ammonium phosphate (16-20-0), and calcium nitrate (15-0-0). Average biomass yields were 10.9 percent greater from fertilizer treatments as compared to the control. Calcium nitrate plots produced 20 percent more biomass. Recently, Thomas (67) reported that nitrogen concentrations up to 112 mg/l in solution increased biomass and rubber yields in greenhouse shrubs. Phosphorus and potash did not significantly influence yields.

Bucks et al. (68) applied nitrogen fertilizer at levels ranging from 33 to 167 percent of recommended rates. Results suggested that to reach high resin and rubber yields in two or three years, moderate-to-high irrigation amounts combined with low nitrogen applications would be required to produce adequate biomass.

Pest control. Oil sprays used in nurseries during 1942 and 1943 were applied for weed control in transplanted stands. Light grades of diesel fuel oil and stove oil were first used in combination with water, and later applied alone (2). Resistant weeds within the rows were removed by hand hoeing or an "in-the-row-cultivator." More recently, preplant and preemergence herbicide treatments have been implemented (69-73). Foster, Ranne, and Moore (74) have applied bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), glyphosate [*N*-phosphonomethyl)glycine], and 2,4-D [2,4-dichlorophenoxy)acetic acid] as broadcast sprays to dormant guayule stands for the control of cool season weeds. Disease, insects, rodents, and their control are the same as for nursery operations.

Conditioning. Shrub conditioning or stressing was practiced during the ERP to increase rubber content and to reduce the moisture content and leaf bulk to facilitate harvesting and transportation (5, 11). Several methods of conditioning were used but the most effective technique was to withhold irrigation water at periodic intervals prior to harvest. Conditioning of dryland tracts was essentially automatic. Recent studies have shown that biomass, resin, and rubber yields increase almost linearly with increasing irrigation, and can be related to the cumulative evapotranspiration (61, 63, 68, 75, 76).

Direct Seeding

The overshadowing expense in stand establishment has been the operation and maintenance of nurseries and greenhouses for the production of seedlings. Pollarding or harvesting the aboveground biomass can, in part, offset a portion of these costs (2). Leaving the roots to resprout

would eliminate the need for reestablishment by transplanting. Nevertheless, the initial planting, plus a subsequent operation after several clippings, would require transplanting. Establishment costs could be reduced with the development of direct seeding techniques.

ERP studies. Direct seeding trials during the ERP were concentrated in the following areas: Salinas Valley, California; Salt River Valley near Phoenix, Arizona; Yuma Mesa, Arizona (true desert conditions); Lower Rio Grande Valley near Raymondville, Texas; Mesilla Valley near Anthony, New Mexico; Coachella Valley near Indio, California; and, San Joaquin Valley, Bakersfield District, California. Taylor (38), in studies near Salinas, California, observed that the germination and emergence behavior of guayule seed greatly limited the following conditions necessary for successful direct seeding: a) minimum daily temperatures not lower than 10° C, b) maximum daily temperatures close to 32° C, c) very shallow cover not exceeding 3 mm, d) no crusting of the soil surface, and e) abundant soil moisture during germination and emergence.

Tingey and Clifford (59) conducted trials in the Salinas and Coachella valleys to determine if the time required to produce satisfactory rubber yields could be shortened by direct field seeding as opposed to transplanting nursery seedlings. Rubber yields from transplants 12 to 18 months after planting were slightly higher than from shrubs that were directly seeded. Therefore, it was concluded that at the current plant spacings (91 x 61 cm and 71 x 51 cm) direct seeding could shorten the planting/harvest period by almost one year. Tingey (77-79) used a Planet Jr. planter to drill dry and pregerminated seed that was either threshed or unthreshed. Sowing in hills and drill rows was done on the edge of irrigation furrows. Emergence was greater when seeds were planted at 3 to 6 mm depths. Dry-sown, threshed seed germinated nearly as well as pregerminated unthreshed seed. Seeding directly in the field on 36 cm rows with no thinning combined with light irrigation, yielded 1,913 kg of rubber/ha at the end of three years (80). A maximum rubber yield of 1,496 kg/ha was obtained 21 months following direct seeding in the Coachella Valley and near the Pacific coastline in California (81).

Taylor (38) summarized the direct seeding experiments in the Salt River Valley near

Phoenix conducted by Helgeman (82). Satisfactory stands were obtained by seeding 3 to 5 mm deep with a loose soil cover. The drill row was located on a shoulder of the seedbed and 2.5 to 5.0 cm above the water level of the furrow. When primary leaves developed on the seedlings, roots had penetrated 20 to 25 cm deep. Therefore, the soil surface could be allowed to become dry, reducing the threat of damping-off. Studies by Davis (83, 84) under true desert conditions in Arizona were also summarized by Taylor (38). Treatments included shading with brush and nursery crops, planting on slopes of ridges, and various irrigation regimes. Fall (September 20 to November 15) and early spring (February 1 to April 15) were the only seasons favorable for seedling establishment. Stands established by direct seeding were better than those established from transplanting.

Cowley (85, 86) sowed pregerminated and dry seed with a Planet Jr. seeder at 6 and 11 kg/ha in south Texas. Emergence was optimum with pregerminated seed planted 3 to 6 mm deep. Taylor (38) reported that studies by Crain and Davis (87) near Anthony, New Mexico, incorporated the use of various nursery crops for shade during germination and emergence but were not successful. Acceptable stands were established by seeding 3 to 6 mm deep in irrigation furrows. September was best for seeding and only one irrigation was required. Climatic conditions in south Texas were generally unfavorable for direct seeding on dryland sites. Trials on fallowed land involved planting in the bottom of furrows to conserve moisture (88). Emergence was suitable only where seed had not been covered too deeply by soil from the furrows.

Recent advances. Planting in the bottom of a shallow furrow (8 to 10 cm deep) cut into the top of the irrigation furrow was the most practical technique used by Whitworth (89). Gel seeding compounded seed handling but reduced the amount of seed required. Whitworth (90) suggested that good seed quality, seedbed preparation, and precision planting were essential for successful direct seeding. Seeds were covered with no more than 3 mm of soil, and if some seed was not visible after sowing, it was planted too deep. A light covering of vermiculite plus a water-absorbent polymer was used as mulch. Initial emergence eight days after planting was

24 plants/m with vermiculite and 10/m for soil cover. Johnson and Fangmeier (91) evaluated hydrogels or water absorbing polymers for use in direct seeding. Soil-incorporated hydrogels produced no beneficial effects, and a surface application inhibited establishment. Fluid drilling, sowing pregerminated seed in a gel carrier, has shown promise for direct seeding (92). Stand establishment was greatest when pregerminated seed was planted 3 mm deep. During germination and emergence water management and disease control was critical. Fluid planting has been compared with dry-sown seed under sprinkler irrigation in west Texas (93). Seedling counts were greatest with dry-sown seed planted in the fall. Gel treatments performed about the same as dry-sown treatments; therefore, the time and extra equipment involved in applying pregerminated seed was not justified. Stand establishment was affected by temperature extremes, heavy rains of short duration, wind, and bird predation. Vermiculite mulch enhanced seed germination and establishment by holding moisture on the beds and preventing surface planted seeds from being washed or blown off site (94). Single rows of about 300 seeds/m were seeded with a Stan Hay vegetable planter on raised beds spaced 1 m on center and 18 m long.

Conditioning techniques (25, 32-34) have markedly improved the planting quality of raw seed. Four planting practices including conditioned seed, pelleted seed, raw seed, and fluid drilling of imbibed seed were evaluated at the Yuma Mesa Experiment Station (95). Maximum percentage emergence of conditioned seed was 65 percent after 30 days, and 34 percent after two months. Bucks et al. (96) compared raw and conditioned seed planted by the following: a) a John Deere planter, b) a Nibex precision planter, c) by hand in pelleted form, and d) a fluid drill. Seedling emergence was generally greatest with fluid drilling since the seeds remained closer to the soil surface for a longer period of time. However, seed spacing was less uniform as compared to the precision planter. Direct seeding was successful when the seeds were conditioned, accurately planted on the soil surface by fluid or precision planting, properly irrigated, and planted at a rate of at least 40/m. Foster et al. (97) seeded raw and conditioned seed at 82 seeds/m with a Gaspardo SV255 pneumatic planter in west Texas. Seedling establishment was

optimum when conditioned seed was sown on the bed surface and covered with vermiculite. Conversely, Foster, Ranne, and Moore (98) reported that in 1988 trials, seedling counts 21 days after planting were highest when seeds were planted 10 mm deep. There was no significant effect on emergence when vermiculite was applied. Rainfall (60 mm) one day after planting washed most vermiculite from the beds, and destroyed the surface planted treatments. Conditioned seed, which can be planted as much as 10 mm deep, was essential for stand establishment. Results from Arizona indicate that conditioned seed can be planted 5 to 10 mm deep, or even 15 mm deep if planting conditions are favorable (99).

Cover crops and synthetic shade covers have been investigated for increasing seedling emergence and survival under sprinkler irrigation. Bucks et al. (100) found that polyshade strips and cloth increased plant survival over no shading when conditioned seed was sown with a precision planter. Shading by wheat increased plant survival in summer and fall 1986 treatments, but not during spring 1987 plantings. Synthetic materials decreased soil, air, and cotyledon temperatures, and increased plant water potentials versus no shade.

Cultural practices. Direct seeding research has emphasized the need for frequent irrigation to promote seed germination, prevent soil crusting and facilitate seedling emergence, and guard young seedlings against desiccation. Following adequate foliar and root development, irrigation should be reduced to avoid damping-off and other diseases prevalent under moist conditions. Frequent water applications, under conventional furrow irrigation, translate to a large water requirement; nonetheless, the soil surface can be kept moist by sprinkler irrigation with lower water inputs (52, 61). Whitworth (90) observed that in direct seeding trials in New Mexico, rainfall caused a secondary flush of germination from seed that previously had failed to germinate under furrow irrigation due to high accumulation of salt. In a separate experiment, additional germination and emergence occurred when furrow irrigation was replaced with sprinklers.

Seedling mortality under furrow irrigation is attributed to leaf and/or root exposure to salt accumulation at the soil surface (101-103). Leaf-induced mortality can be magnified when

wind-damaged seedlings are exposed to saline splatters during light showers. Whitworth (89) determined that salt buildup was the primary factor responsible for stand reduction following emergence. The greatest survival (28 percent) occurred when the irrigation furrows were kept full of water; thus, the salts were kept diluted, as compared to repeated drying out and watering. Seedling emergence averaged 57 percent in field plots near El Paso, Texas, furrow irrigated with 0.8 dS/m water, but only 17 percent with water of 4.5 dS/m (104). Seedling survival was zero in the 4.5 dS/m treatments. Water of low salinity and irrigation methods that minimize surface salt accumulation were recommended for acceptable seedling emergence and establishment. Sprinkler irrigation minimizes salt collection on the soil surface and appears to be better adapted to direct seeding than furrow irrigation (61). However, salinity levels contributing to mortality from foliar salt absorption have not been determined.

Successful stand establishment with sprinkler irrigation has been reported, particularly with conditioned seed (94-98). Zittlosen and Fangmeier (105) tested the effect of sprinkler and drip irrigation on direct seeding at Tucson, Arizona. Average stand survival was 52 percent (85 seedlings/m) under sprinklers. Establishment required 13.7 mm/day (410 mm total) of water for the first 30 days. Air temperatures of 34° to 36° C were not adverse, but average soil water contents below 9.3 percent were inhibitory. Drip irrigation used 55 percent more water (635 mm) and resulted in only one-half the survival rate compared to sprinkling. Gonzalez and Rektorik (106) examined the use of drip irrigation for germination and seedling establishment under dryland conditions in south Texas. Seeds were planted at approximately 80/m with a Gaspardo pneumatic planter. Treatments included no supplemental water, 18 mm applied over five days, and 36 mm added over a 10-day period following seeding. Seedling counts ranged from less than 1/m with no water to 7/m in the 36-mm treatment.

Cultivation is not feasible until the seedlings become established and the furrows are dry enough to support equipment. Machinery involves adapting customary implements used in other row crops of the area.

Fertilization requirements should be based on soil fertility and general conditions of the plants. Nitrogen was routinely applied to nursery seedlings under ERP operations. To avoid additional weed competition problems, fertilization could be applied well after establishment or the following growing season if needed. Bucks et al. (96) anticipated that optimum water and nitrogen applications over a three-month establishment period would be at least 300 mm of water and 56 kg/ha of nitrogen.

Weed control in direct-seeded guayule has been successful with various preplant and preemergence compounds. No herbicides have currently been registered for use in guayule production. Whitworth (73, 107) and Boyse, Whitworth, and Clark (108) tested several herbicides for their phytotoxicity to direct-seeded guayule. Only DCPA (dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate), and pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] demonstrated adequate selectivity. Greenhouse experiments have shown that trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)benzenamine] and pendimethalin at rates of 0.56 to 1.68 kg/ha were the most promising for preemergence weed control, but some guayule toxicity was evident (71). Complete guayule mortality occurred with diuron [*N*'-(3,4-dichlorophenyl)-*N,N*-dimethylurea] and simazine [6-chloro-*N,N*'-diethyl-1,3,5-triazine-2,4-diamine]. Other pest control problems such as disease, insects, and rodents are similar to those covered in nurseries and transplants.

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Chapter 7

Water and Nutrient Requirements of Guayule Under Irrigated and Dryland Production

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INTRODUCTION

Domestication of guayule (*Parthenium argentatum* Gray) in the United States will occur in environments that are likely to be far different from the plant's natural conditions. Climatic conditions encompass the following: a) moderate temperature and rainfall in California, b) hot, dry, semiarid conditions in Arizona, c) desert-like environments resembling guayule's native habitat in southwestern Texas, and d) humid, warm conditions in south Texas. The northernmost boundary of the shrub's natural distribution extends to the Fort Stockton, Texas, area (Chapter 9, Figure 1). The potential growing regions have calcareous to acidic soils that are most likely different from the native habitat's calcareous soils. Similarly, rainfall will differ from the 125 to 350 mm of the native area; furthermore, seasonal distributions will be different. For our discussion, dryland or nonirrigated systems will be primarily in the 400 to 500 mm rainfall area with favorable climatic conditions that can sustain growth and rubber production. Rainfall in the irrigated system will be less than 300 mm with water available for supplemental application. Supplemental water use in dryland systems will be limited by availability and cost; it would be used primarily for plant establishment.

Cultural practices for the two systems would be different. Plant growth patterns and yields will depend upon environmental factors, and information is needed on the best management

practices to optimize rubber yield and economics. In the 1980s investigations on water and nutrient requirements of guayule under irrigation have been conducted primarily in central and western Arizona at Mesa, Tucson, Yuma, and Phoenix. Investigations of guayule under dryland conditions have been conducted in west Texas at Fort Stockton and El Paso, and in south Texas at Rio Grande City. These regions are probably the extremes in climatic range relative to water availability. In terms of water quality, research has been done at Brawley, California, and El Paso, Texas. To incorporate the available water-nutrient-salinity studies, we still must refer back to the research results of the 1940s, which were obtained mainly at Salinas, California, under the Emergency Rubber Project (ERP).

During the ERP, a set of recommended standards was proposed to address irrigation water requirements for the various climatic conditions where guayule had a potential for cultivation (Table 1). Three possible zones for guayule cultivation were designated—an arid zone, an intermediate zone, and a coastal zone. Criteria for irrigation water quality were also suggested and are listed in Table 2. The original data sets have been updated with information obtained in the 1980s.

It is known that either too little or too much water affect germination and survival of young directly seeded plants and young transplants (2). An abundance of water can cause problems with disease, soil aeration, and weed competition—problems that detrimentally affect plant growth and yield. Excess water is harmful to guayule plants of all ages, but much more so to the young plants, especially during the hot summer months (3). Under irrigation, the growing period before shrub harvest can be shortened and the rubber yields increased. Kelley (4) indicated that where temperature conditions are suitable for guayule, soil water is probably the most important factor for plant growth and rubber accumulation. In the desert environment, growth is slow and is controlled by water availability. This chapter will be directed toward water requirements and management of guayule. Other factors that may closely interact with them will also be considered.

Table 1. Annual water requirements for guayule production.

Suitability class		Arid zone (mm)	Intermediate zone (mm)	Coastal zone (mm)
		Water quantity		
I.	Good	1,500 (910)	1,200 (760)	900 (520)
II.	Permissible	1,200-1,500 (760-910)	900-1,200 (610-760)	600-900 (400-520)
III.	Doubtful	900-1,200 (600-730)	600-900 (460-600)	300-600 (300-370)
IV.	Unsuitable	<900 (<580)	<600 (<430)	<300 (<270)

Note: Updated data of McGinnies and Mills (1), in parenthesis, and 1989-based information. Intermediate zone has late spring rains, and coastal zone has fog and cooler weather compared to the arid zone.

PLANT WATER STRESS CONCEPT

Investigators have known for a long time that stress plays an important role in rubber production (2). More recently, analysis of rubber transferase enzymes shows that their activity is higher when the plant is under water stress (5, 6). The latter researchers observed a twofold increase in rubber transferase activity when plants were subjected to stress. Other field studies

Table 2. Water quality for guayule production.

Suitability class	Total dissolved solids (dS/m)	Sodium (%)	Boron (ppm)	Chloride (ppm)	Sulfate (ppm)
I. Good	<1.5				
	(<0.82)	(<40)	(<2.0)	(<250)	(<340)
II. Permissible	1.5-3.0				
	(0.82-2.2)	(40-60)	(2.0-3.0)	(250-425)	(340-575)
III. Doubtful	3.0-6.0				
	(2.2-3.3)	(60-80)	(3.0-3.8)	(430-710)	(580-960)
IV. Unsuitable	>6.0				
	(>3.3)	(>80)	(>3.8)	(>710)	(>960)

Note: Updated total dissolved solids data of McGinnies and Mills (1), in parentheses, and 1989-based information. No late information is available for sodium, boron, chloride, and sulfate.

have shown that water stress has a strong effect on rubber accumulation. But, in the practical situation, maximizing rubber synthesis must be balanced with other important plant functions such as seed and biomass production. The plant needs to build up storage cells where the rubber can accumulate. It also must produce vigorous seeds, which are essential for guayule commercialization. For example, two seed collections may be possible in dryland culture, whereas three to seven seed collections could be made under irrigation depending upon climatic conditions and irrigation frequency (1).

Benedict, McRary, and Slattery (7) imposed various combinations of moisture stress on guayule and found that plants growing under alternating periods of low and high soil moisture

conditions had more rubber per plant than those grown under either continuous low or continuous high stresses. Timing of stress was also important so that stress imposed during the spring, when growth and rubber accumulation were the greatest, resulted in the highest gain in rubber. Stress effects were not observable in the winter months. Veihmeyer and Hendrickson (8) noted a marked increase in rubber content during the winter, regardless of the soil moisture status.

The crop water stress index (CWSI) concept, previously used for many field crops, was adapted to guayule by Nakayama and Bucks (9). Ray et al. (10) used this technique for scheduling water application for maintaining various irrigation treatments. Remote infrared thermometry measurements of crop temperatures and a knowledge of the water vapor content (relative humidity) of the atmosphere provide the means for relating plant water stress to the soil water status. Plant yields were found to be negatively and linearly related to the computed CWSI range of 0.2 for the low stress to 0.75 for high stress (11). Garrot, Ray, and Fangmeier (12) also found good inverse correlation between rubber yield and seasonally averaged CWSI. The CWSI could also be linearly related to soil water content and evapotranspiration (11).

Leaf temperature is a manifestation of the physiological status of the plant. Allen and Nakayama (13) have shown that the CWSI could be related to the leaf water potential, osmotic potential, and stomatal conductance of the leaf. These relations were of particular interest because at a CWSI value of 0.6 and above, the plant could no longer maintain turgor through osmotic adjustment. Unfortunately, CWSI measurement is difficult to make in young plants (which require the most attention relative to water management) because of the lack of complete canopy cover at this stage of growth.

Photosynthesis for biomass and rubber formation continued in guayule even though the plant was undergoing moderate stress (14). Rapid recovery from a low level of photosynthesis occurs once the water stress is relieved with irrigation. In nature, rainfall in adequate quantities will do the same thing. The relative leaf water content also followed the plant water stress status and the soil water deficit (15). Thus, the CWSI approach provides a very useful tool for monitoring and managing water stress in the guayule plant.

Water use and photosynthetic behavior of guayule are strikingly different from other domesticated crops. For example, Downes and Tonnet (16) found in phytotron studies that four-year-old potted guayule transpired 0.9 $mmol/m^2/s$, whereas wheat transpired about twice or 1.6 $mmol/m^2/s$ on a leaf area basis. They suggested a coupling of low transpiration rates to low photosynthetic rates. Field measurements and computed estimates indicate that the photosynthetic efficiency of guayule is about one-third to one-tenth that of other crops (17). Guayule grown under irrigation could reach maximum photosynthetic rates approximately seven times higher than plants grown under dry regimes.

WATER AND NUTRIENT REQUIREMENTS

Plant Establishment

Germination of seeds and survival of seedlings and transplants are highly dependent upon an adequate soil water supply during the establishment period. Lack of water has been emphasized as a factor in the failure of seed germination, but equally as important is the excess of water which causes disease and aeration problems both in the nursery and in the field. Waterlogging is particularly harmful during the warm summer period (3). The general solution for plant establishment is to keep the media moist, but not wet.

Dryland cropping system. Frequent irrigation is needed for transplant survival, but is not always possible under dryland conditions. To avoid transplant loss, Gonzalez and Rektorik (18) used large seedling containers (10 x 10 x 10 cm) so that an adequate root volume developed before seedlings were transferred to the field. Survival varied with the season with 85 percent occurring in the fall and 51 percent in the spring plantings. Seedling survival was further increased by adding 400 ml of water per plant. Milthorpe (19) obtained good survival under dryland conditions when the transplant was made in moist soil.

Gonzalez and Rektorik (20) also directly seeded guayule under dryland conditions. At a planting rate of 72 seeds/m and after 50 days, a 0.6 plant/m survival was achieved without supplemental irrigation; and, with trickle irrigation of 3.6 cm over the first 10 days, establishment increased to 6.7 plants/m.

Irrigated cropping system. Smaller transplanting potting cells can be used for seedlings in irrigated systems than for those in dryland systems. In the ERP procedure, bare-root transplants were also used (2). Bucks, Nakayama, and French (21) applied up to 250 mm of water with furrow irrigation followed by sprinkler irrigation spaced over a three-month establishment period to achieve 95 percent transplant survival. A 98 percent survival was obtained with 380 mm in another experiment (22). Fangmeier et al. (23) estimated establishment water applications to be about 500 to 630 mm. Frequent 20-mm weekly water applications were used by Miyamoto, Piela, and Gobian (24) in furrow plots for a five-week period to obtain 95 percent survival.

Plant establishment by direct seeding also requires careful water management. Zittlosen and Fangmeier (25) sprinkler irrigated seedbeds on a daily basis with cycling throughout the 24-hour period for 30 days to maintain a visibly moist soil surface; they obtained a 52 percent plant establishment. In an earlier study, only about one-half the establishment rate with the sprinkler irrigation was attained by drip irrigation, which used 55 percent more water over a 27-day period. Saline water affected the survival of seedlings much more than it did transplants. A detailed summary of the methods and amounts of water used for plant establishment is presented in Table 3.

Directly seeded plants are extremely sensitive to salinity, and mortality is caused by damage to the hypocotyl due to the buildup of salt at the soil surface (27). Planting seeds to a depth of 10 mm or more enhances seedling survival and establishment (29). This practice is in contrast to the early recommendation that shallow seed placement was the best (30). Seed treatment with hypochlorite was practiced in the ERP era and as recently as the early 1980s. Seeds are now being treated with a combination of gibberellic acid, polyethylene glycols and fungicides

Table 3. Effect of the quantity and quality of water on guayule establishment.

Planting method	Irrigation interval (day)	Duration (day)	Total Water application (mm)	Salinity (dS/m)	Survival (%)
Transplant	7	35	100	0.8	95[a]
	3-7	49	250	1.2	95[b]
	7	35	100	4.6	95[a]
	7	35	100	7.2	79[a]
Seed	0.5	35	500	0.8	10[c]
	1-3	60	250	1.4	34[d]
	1	30	410	0.4	52[e]

Source: Miyamoto and Bucks (26).

[a]Miyamoto, Piela, and Gobian (24).
[b]Bucks, Nakayama, and French (21).
[c]Miyamoto et al. (27).
[d]Bucks et al. (28).
[e]Zittlosen and Fangmeier (25).

to improve seed germination, vigor and resistance to pathogenic organisms (31, 32). Shading, and particularly the use of mulch to prevent surface crusting together with deeper planting, have also improved plant establishment by direct seeding (33). Optimum seedling establishment was obtained by Foster et al. (34) by planting conditioned seed at a 10-mm depth with a vermiculite mulch cover which improved the soil moisture condition around the seed and prevented loss of seeds from the bed by water and wind.

Clipped Plant Yields

Clipping studies initiated in the 1940s were not completed because of budgetary constraints (35). The consensus in that period was to grow guayule on an eight-year cycle with shrub clipping after five years growth. Under present-day economics, this does not appear to be feasible. The main reasons for clipping were to avoid replanting and related costs, and to obtain a larger biomass in a shorter interval for the succeeding ratoon crop.

Dryland cropping system. Gonzalez (36) conducted studies comparing yields of shrubs clipped at yearly intervals to whole plants harvested over a three-year period. Little difference was observed in rubber and resin content in the clipped versus whole-plant harvest. Rubber production is compared in Table 4. In the clipped treatment, the highest plant population as

Table 4. Rubber yield from clipped and whole guayule shrubs over a three-year interval, Rio Grande City, Texas.

Density plants/ha	Rubber yield, kg/ha					
	First clipped plants			Whole plants		
	1983	1984	1985	1983	1984	1985
55,500	364a[a]	517a	916a	436a	689a	923a
42,000	296ab	411a	613b	279a	620a	874a
32,000	255b	460a	659b	296a	296a	830a

Source: Gonzalez (36).

Note: Transplanted September 1981.

[a]Values followed by the same letters are not significantly different at the 5 percent level according to the Duncan's Multiple Range test.

Table 5. Cumulative rubber yields accounting for clipping and shrub regrowth, Rio Grande City, Texas.

Density plants/ha	Rubber yield, kg/ha		
	Two clippings[a]	One clipping[b]	Single harvest[c]
55,500	672a[d]	720a	923a
42,000	677a	600a	874a
32,000	534a	629a	830a

Source: Gonzalez (36).

Note: Transplanted September 1981.

[a]First clipping (February 1983); second clipping (1983 to 1984, top regrowth), and final harvest (1984 to 1985, top regrowth plus root).

[b]First clipping (February 1984) and final harvest (1984 and 1985, top regrowth plus root).

[c]Whole-plant harvest (February 1985) without previous clipping.

[d]Values followed by the same letters are not significantly different according to the Duncan's Multiple Range test.

compared to the two lower, gave significantly higher rubber yields. While the yields in the whole-plant harvest relative to population were not significant, the same trend in the data occurred. Cumulative yields, which include clipping and regrowth, are presented in Table 5. According to these sets of data, no difference due to method of harvest was present. Whole-plant harvest was recommended every three years for obtaining higher yields and best economics under dryland culture.

Irrigated cropping system. Data for the relationship between rubber yields for clipped and whole-plant harvest at Yuma, Arizona, are presented in Table 6. A central composite rotatable statistical design was used which permitted the testing of several irrigation and nitrogen

Table 6. Cumulative rubber yields accounting for clipping and shrub regrowth over a four-year period, Yuma, Arizona.

Treatment	Water[a] (%)	Nitrogen[b] (%)	Rubber yield, kg/ha One (clipping)[c]	One (clipping[d])	Single (harvest[e])
T1	60	100	1,525ab[f]	1,315a	1,535a
T2	65	53	1,835ab	1,360a	1,580a
T3	65	147	1,615ab	1,350ab	1,615a
T4	100	33	1,440a	1,700ab	1,370a
T5	100	100	2,120ab	1,925a	2,270cd
T6	100	167	2,320b	1,970b	2,045bc
T7	135	53	1,710ab	1,645ab	1,945b
T8	135	147	2,080ab	2,000b	2,480d
T9	150	100	1,980ab	1,765ab	1,985bc

Source: Bucks et al. (22).

Note: Transplanted January 1982, 49,500 plants/ha. Includes unpublished data for 1986 harvest.
[a]100 percent represents irrigation to match evapotranspiration.
[b]100 percent represents 56 kg/ha of nitrogen applied twice each year.
[c]First clipping (January 1984) and final harvest (January 1986, top regrowth plus root).
[d]First clipping (January 1985) and final harvest (January 1986, top regrowth plus root).
[e]Whole-plant harvest (January 1986) without any clipping.
[f]Values followed by the same letters in the column are not significantly different at the 5 percent level according to the Student-Newman-Keuls' test.

application levels in addition to harvesting techniques (22). Yields for the single whole-plant harvest after four years of growth were the same as the following: a) clipping at two years of shrub growth plus whole-plant harvest after two years of regrowth, and b) clipping after three years of growth plus whole-plant harvest after one year of regrowth. Within a set (column), yields were highest with the higher water-nitrogen combination. High water applications were possible because of the good drainage of the 95 percent sand Superstition soil at Yuma, Arizona.

Garrot and Ray (37) reported in their Tucson, Arizona, studies that the cumulative rubber production of two succeeding sets of two-year clippings was 71 percent higher than that of a single four-year-old whole-plant harvest. Similarly, clipping two-year-old plants plus whole-plant harvest after one year of regrowth yielded 64 percent more rubber per plant than the unclipped whole-plant harvest of the three-year-old plant. Results of the succeeding clipping experiment of Ray et al. (10) in Table 7 did not show the positive effects of clipping reported earlier. Rubber content was not especially different with clipping, nor irrigation levels. Of particular note is the difference in survival of the 1985 clipping (average of 66%) versus the survival in the 1984 clipping (average of 98%) which could present a problem with clipping culture. Hoffman et al. (38) with plantings at Brawley, California, had difficulties in regrowth after clipping when the salinity of the irrigation water was above 6.5 dS/m. Regrowth was about 90 percent with 1.2 dS/m water, whereas it dropped to about 70 percent with 3.2 dS/m, 20 percent with 6.5 dS/m, and 0 percent with 9.4 dS/m. However, for the irrigation treatments that were not affected by salinity, Hoffman et al. (38) got greater growth and biomass production as a result of clipping.

Whole-Plant Yields

Tingey (39) made an extensive study on the interaction of fertilizer x irrigation level x plant density at Salinas, California, with an annual precipitation of 330 mm. His findings include the

Table 7. Cumulative biomass production of clipped and whole guayule shrubs, Tucson, Arizona.

Water treatment	Fresh weight (g/plant)			Rubber content (%)			Plant survival (%)	
	T[a]	R[b]	C[c]	T[a]	R[b]	C[c]	1984	1985
Wet[d]	53	1,308	1,162	4.2	3.6	3.9	98	61
Medium[e]	608	962	1,022	3.7	3.0	5.1	98	73
Dry[f]	463	726	854	3.9	2.9	3.7	98	66

Source: Ray et al. (10).

Note: Transplanted October 1982, 36,650 plants/ha.
[a]Twice clipped plants (March 1984 and March 1985).
[b]First clipping (March 1984) and final harvest (March 1985, top regrowth plus root).
[c]Whole-plant harvest (March 1985) without clipping.
[d]Irrigation when CWSI exceeded 0.3.
[e]Irrigation when CWSI exceeded 0.6.
[f]Irrigation when CWSI exceeded 0.9.

following: a) fertilizer did not affect rubber yield of three-year-old plants; b) highest yields were obtained with the "light" irrigation treatment of 5 irrigations in the two-year period after establishment compared to the "heavy" with 11 and the "none" with only 1; and c) yields were highest with the highest plant population of greater than 300,000 plants per hectare (our estimate on population). The highest yield obtained was 1,900 kg/ha for the 33-month-old shrub. The soils in the Salinas area, preceding guayule culture, were fertile soils and highly fertilized for vegetable production so that plant response to nitrogen fertilizer would be

minimal. Hammond and Polhamus (2) cite a study at San Clemente, California, which has a climate similar to Salinas. Here the yield was 1,500 kg/ha produced by 21-month-old shrub from direct seeding with dense planting and light irrigation, but no fertilization. With these experiments in mind and considering the annual rainfall of 330 mm, the results can be related to the studies conducted under different climatic conditions in the 1980s.

Dryland cropping system. Except for supplementary water application for plant establishment, water maintenance of the shrub will depend entirely on natural rainfall. Reasonable yields can be obtained in the 650-mm rainfall areas. Water used by the guayule plant must be characterized to establish a baseline for comparison with crop production in other regions, particularly where irrigation is to be practiced. Water-use efficiency—the production of usable products such as biomass, grain, rubber, etc., per unit of water used by the plant—can be utilized for assessing the agricultural productivity of the crop. Yield data of Gonzalez (36) and other unpublished information are listed in Table 8. Water-use efficiency calculations were based on total rainfall because data on soil water depletion were not available. The high density planting tended to show higher water-use efficiency than the lower population densities as would be expected from the yield data of Table 5. Assuming a water-use efficiency value of 0.04 kg/m^3 for rubber and shrub rubber content of 5 percent, the biomass production of 0.8 kg/m^3 is obtained, which is similar to that given by Bucks et al. (40) for the irrigated cropping system. Analyses of dryland cropping in Australia (Chapter 15, Table 1) give water-use efficiencies of 0.019 to 0.046 kg/m^3 for rubber, and 0.17 to 0.35 kg/m^3 for biomass. Again, the water-use efficiencies of rubber are similar in the various locations. The values for biomass production are lower in Australia, but the rubber values are similar since the reported values for rubber content are in the 11 percent range, whereas, those in Texas and Arizona have been in the 5 percent range.

Miyamoto and Bucks (26) compared yields with various aspects of water quantity and quality and computed water-use efficiencies for the combinations available. Some of their results are summarized in Table 9. Salinity of the irrigation water decreased the rubber yield

Table 8. Water-use efficiency for the production of rubber, Rio Grande City, Texas.

Plant density (plants/ha)	Water use efficiency, kg/m^3				
	Clipped-plant harvest		Whole-plant harvest		
	One clipping[a]	Two clippings[b]	month 17[c]	29[d]	41[e]
55,500	0.038	0.043	0.067	0.056	0.053
42,000	.040	.034	.038	.050	050
32,000	.030	.036	.045	.053	.048

Source: Gonzalez (36).

Note: Transplanted September 1981.
[a]First clipping (February 1983), second clipping (1983 to 1984, plus top regrowth), and final harvest (1984 to 1985, top regrowth plus root).
[b]First clipping (February 1984), and final harvest (February 1984 and 1985, top regrowth plus root).
[c]Rainfall 655 mm from planting to harvest.
[d]Rainfall1,240 mm from planting to harvest.
[e]Rainfall 1,740 mm from planting to harvest.

and water-use efficiency. Water quality criteria developed in the 1940s (Table 2) indicated that salinity greater than 3.3 dS/m would be unsuitable for guayule culture. However, Maas et al. (41) in experiments at Brawley, California, observed that guayule was more salt tolerant than many crops that were considered to be salinity tolerant at the 6 dS/m level. The apparent discrepancy in the salinity tolerance results between the Texas and California locations could be explained by the high sodium content in the Texas water versus the high calcium content in the California water. Wadleigh and Gauch (42) in greenhouse sand culture noticed the detrimental effects of high sodium concentrations compared to calcium on guayule growth. Other

Table 9. Water-use efficiency for the production of rubber at different irrigation and water quality levels.

Irrigation response, % or salinity (dS/m)	Measured ET (mm)	Rubber yield (kg/ha)	Water-use efficiency (kg/m^3)
56.0[a]	2,190	844	0.038
36.0	1,470	590	.040
11.0	960	560	.058
0.8[b]	1,960	610	031
4.6	1,690	560	.033
7.2	1,280	350	.027

Source: Miyamoto and Bucks (26).

[a]Percentage available water stored in root zone before irrigation (salinity at 0.8 dS/m).
[b]Water salinity.

observations on the effect of salinity by Maas et al. (41) indicated the following: a) that guayule was highly salt tolerant after plant establishment; b) that threshold soil salinity was 7.8 dS/m; and c) that salt threshold did not affect rubber content in contrast to earlier findings. Yields were not affected when plants were irrigated with 3 dS/m salinity, but were reduced at 6 dS/m and higher. Plant mortality increased with time by using irrigation water above 6 dS/m quality water.

Hoffman et al. (38) investigated the interactions of salinity x plant population and found no statistical difference between these factors. However, there appeared to be a tendency for decreased yields as the population increased from 27,000 to 81,000 plants/ha.

Irrigated cropping system. Extensive research has been conducted on irrigated guayule by Bucks and co-workers at Mesa (21, 40, 43, 44) and Yuma (22), Arizona. Their studies show that even though guayule is a drought-tolerant plant, irrigation can greatly increase its growth rate and rubber yield compared to plants growing in their native habitat. For the Mesa experiments, six levels of irrigation were used (Table 10) with 15 periodic whole-plant samples taken over a continuous four-year period to monitor rubber accumulation with plant age, water application, water use, and other plant physiological parameters. The rate of rubber accumulation over the four-year period is illustrated in Figure 1 for the various irrigation treatments. The results show the presence of a seasonal and continuous accumulation of rubber for all of the different irrigation treatments. The yearly optimal harvest periods are designated by dashed lines in the figure and occur in the late winter to early spring months. Rubber concentrations

Table 10. Irrigation water application and evapotranspiration (ET) for four-year-old guayule, Mesa, Arizona.

Irrigation treatment[a]	Number of irrigations	Irrigation and rainfall (mm)	Measured ET (mm)
I_1, Irrigate at 60% soil water depletion (wet)	43	7,950	7,040
I_2, Irrigate at 70% soil water depletion (wet)	35	6,530	5,810
I_3, Irrigate at 80% soil water depletion (medium)	28	5,720	5,130
I_4, Irrigate at 90% soil water depletion (medium)	23	4,750	4,390
I_5, Same as I_4, but delay for two weeks (dry)	18	3,950	3,700
I_6, Same as I_4, but only 3 irrigations/year (dry)	12	2,960	3,240

Source: Bucks et al. (43).

[a]Soil water depletion is based on the percentage of available soil water, which is defined as the water between "field capacity" and "wilting point."

were significantly higher by about 1 percent in the dry I_6 than the wet I_1 treatment for all harvests, but the higher rubber content did not compensate for the lower biomass of the dry treatment in terms of rubber yields. The effect of irrigation levels on total rubber yield is obvious from the figure and the results given in Table 11.

In the dry treatments, water from the soil profile made up for evapotranspiration over and above that applied by irrigation and rainfall (columns 3 and 4 of Table 10). Roots were able to extract the soil water down to 3 m depth as determined by soil water content measurements and root samplings. For the second-year harvest, yields were similar in irrigation treatments I_4 through I_6 and could possibly be caused by root absorption of soil water still present at greater depths. The dryland rubber yields of Table 4 follow the dry I_6 treatment when they are compared to each other in Figure 1.

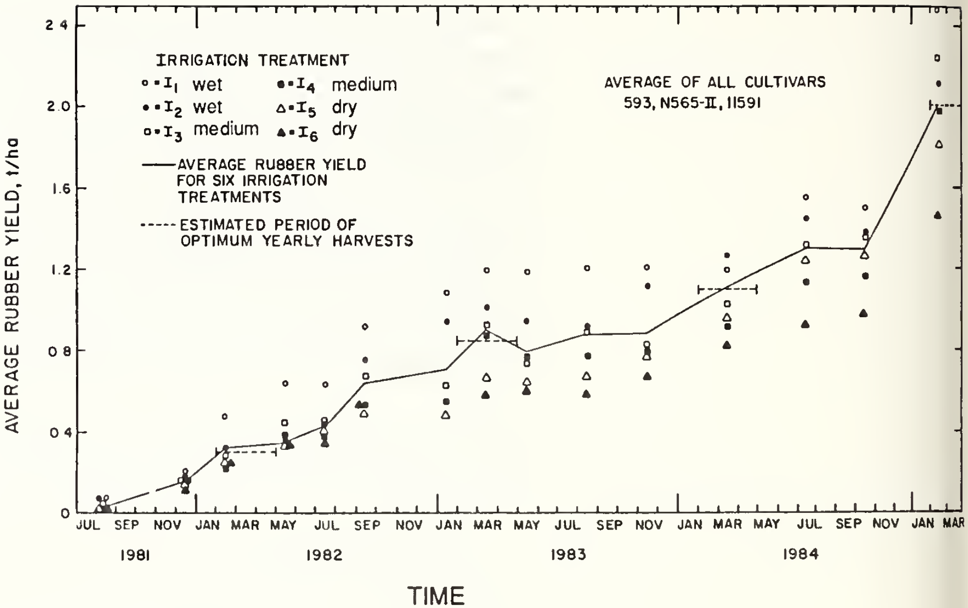

Figure 1. Rubber yields averaged for cultivars 593, N565-II and 11591 for 15 successive whole-plant harvests under six levels of irrigation and 54,000 plants/ha, Mesa, Arizona. (After Bucks, Nakayama, and Allen [45].)

Water-use efficiencies for the dry treatments are higher than for the wet treatments. Although statistical comparisons cannot be made between the dryland and irrigated cropping systems (Tables 8 and 11) similar trends are noticeable in both cases. In the dryland situation, the soil water depletion in the high plant population would be expected to be greater than the low population, and consequently, its water-use efficiency would be higher.

Table 11. Guayule rubber yield and water-use efficiency for two- and four-year-old shrubs, Mesa, Arizona.

Irrigation treatment	Second year: Rubber yield (kg/ha)	Second year: Water use efficiency (kg/m³)	Fourth year: Rubber yield (kg/ha)	Fourth year: Water use efficiency (kg/m³)
I_1, wet	1,160a[a]	0.038	2,530a	0.035a
I_2, wet	940a	.038	2,114c	.036a
I_3, medium	630b	.032	2,250b	.044ab
I_4, medium	560b	.032	1,990cd	.045ab
I_5, dry	500b	.034	1,820d	.049b
I_6, dry	600b	.038	1,470c	.045ab

Source: Bucks et al. (44).

Note: Transplanted April 1981, 54,000 plants/ha. Yield averaged for cultivars 593, 11591, and N565-II.
[a]Values followed by the same letters in the column are not significantly different at the 5 percent level according to the Duncan's Multiple Range test.

Water-use efficiency (WUE, kg/m³) can be related to cumulative evapotranspiration (ET, mm) as shown in Figure 2 and can be further described by the function:

$$WUE = 4.82 \times 10^{-2} + (1.20 \times 10^{-6})\, ET - (4.61 \times 10^{-10})\, ET^2,$$
$$R^2 = 0.81,$$

where R^2 is the regression coefficient.

It is evident that water-use efficiency decreases with higher levels of water application. However, the primary objective is to increase the economic rubber yield over attainment of

maximum water-use efficiency. The ordinate scale can be converted into units of yield per water applied so that the values 0.03, 0.04, and 0.05 kg/m^3 become 3.0, 4.0, and 5.0 kg/ha-cm of water (6.6, 8.8, and 11.0 lb/ac-in), respectively.

Rubber yield (Y, kg/ha) can also be related to the cumulative evapotranspiration (Figure 3) in the 3,000 to 7,000 mm range by the expression

$$Y = 847 + 0.242\ ET, \quad R^2 = 0.87.$$

The yields shown in these examples are higher than any obtained before. The leveling of yields with water application rates observed with other crops was not achieved in this set of studies.

And, rubber yield (Y, kg/ha) can be related to the physiological factor of seasonally averaged crop water stress index (CWSI) by the equation

$$Y = 2857 - 1744\ CWSI, \quad R^2 = 0.93$$

and similarly, ET (mm) by

$$ET = 8050 - 6666\ CWSI, \quad R^2 = 0.95.$$

Thus, measurable environmental or physiological parameters such as ET and CWSI can be used to predict rubber yield once they are established for the site, plant population, and cultivar.

For the irrigation study at Yuma, Arizona, five levels of water application in the range of 50 to 150 percent of ET and 33 to 167 percent of recommended nitrogen levels (two 56 kg/ha applications per year as ammonium nitrate) were used as previously described in Table 6. The clipping and whole-plant rubber yields are compared in the table. An example of the rubber yield contour lines for the four-year-old whole-plant harvest is given in Figure 4. Maximum rubber yields were obtained in the 100 to 125 percent water and 100 to 125 percent nitrogen

application ranges. The yield (Y, t/ha) can be related to water (W, m) and nitrogen (N, kg/ha) application by the following equation:

$$Y = -1301 + 515.2\,W + 5.346\,N - 0.3896\,W^2 - 0.007226\,N^2 + 0.2976\,W{\cdot}N, \quad R^2 = 0.93.$$

The plants responded best to nitrogen under high water application levels. Rapid growth and large biomass were attainable in the well-drained soil.

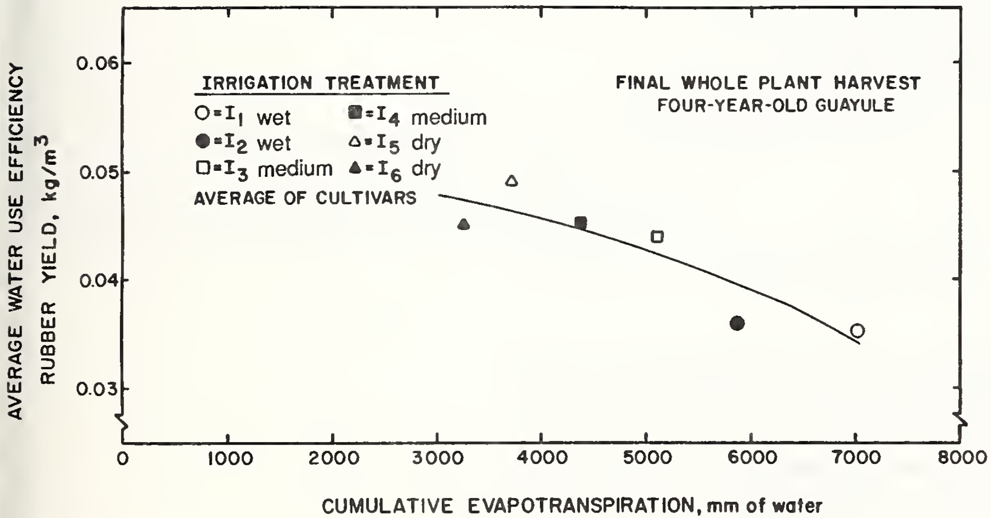

Figure 2. Relationship between water-use efficiency and cumulative evapotranspiration for four-year old guayule, Arizona. (After Bucks et al. [43].)

Other field studies looking at the irrigation-fertilizer interactions have been initiated (45, 46), but complete data are not available at this time. Rubis (46) conducted an experiment at Marana, Arizona, to determine the response of guayule to one nitrogen fertilization level of 112 kg N per ha per year. He found a 20 percent yield increase with calcium nitrate and only 11 percent increase with urea and ammonium phosphate sources. Cannell and Youngner (45) at Riverside, California, applied very low nitrogen fertilizer rates (18 kg/ha maximum) at six irrigation levels and got responses in the larger plants. No yield data were available in these studies.

SUMMARY

Higher guayule rubber yields and shorter growing cycles are attainable under irrigated than under nonirrigated cropping systems. However, these may not be the final governing factors because more dryland than irrigated land is available for cultivation and operational costs are expected to be less in dryland than in irrigated agriculture. Water quantity (rainfall or irrigation) and salinity (total salt concentration and type) are important factors which must be considered in site selection. Salinity threshold and yield decrements with increasing salt content have been developed, showing that established guayule is more salt tolerant than other crops such as cotton. Plant establishment requires an adequate source of water. The controlled application of water to maintain a suitable environment for seedling growth is important. Excess irrigation must be avoided.

The guayule plant, although drought tolerant, grows exceedingly well with supplemental irrigation. The plant exhibits its own distinct physiological responses to water stress which can be identified and used for water management. With existing guayule cultivars under dryland culture, an annual rubber yield of at least 200 kg/ha/y can be expected with rainfall in the 400 to 500 mm range; and, under irrigated systems, rubber yields of over 500 kg/ha/y can be

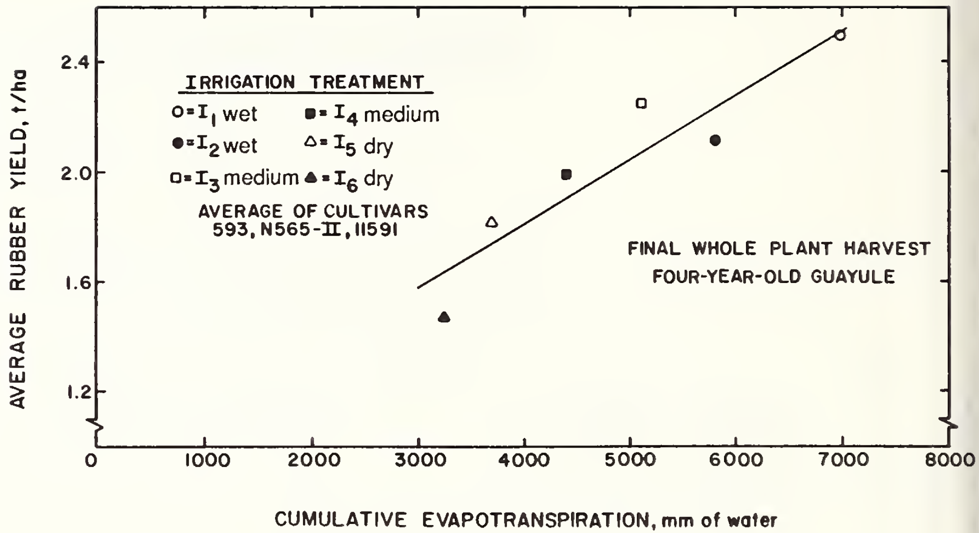

Figure 3. Relationship between rubber yield and cumulative evapotranspiration, Mesa, Arizona. (After Bucks et al. [43].)

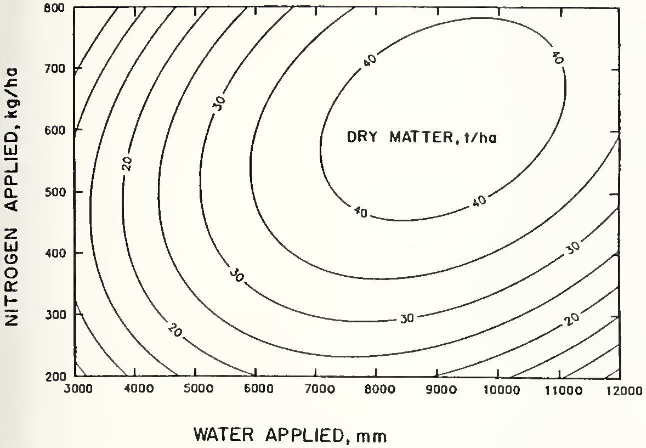

Figure 4. Rubber yield contour lines for whole-plant harvest of four-year-old guayule averaged for cultivars 593, N565-II and 11591, Yuma, Arizona. (After Bucks et al. [22].)

attained with water applications (irrigation + rainfall) of 1,000 to 1,300 mm. The plant does not require high nutrient levels, except where high water application levels are imposed. Information is not complete regarding the benefits of clipping versus whole-plant harvests. Additional long-term experiments must be conducted. Limited studies indicate that yields can be increased by increasing plant population density. The upper limit has not been determined. Successes in direct seeding should provide the means for attaining the high plant densities. Nutrient (nitrogen) requirements of guayule have not been fully resolved, but unlike other crops the plant does not respond as readily to fertilization. Nutrient deficiency may not be a problem because guayule growth is slow so that the rate of soil nutrient availability is not exceeded by root absorption.

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Chapter 8

Plant Health: The Interactions of Guayule, Microorganisms, Arthropods, and Weeds

J. D. Mihail, Stanley M. Alcorn, and J. Wayne Whitworth

The successful production of guayule will ultimately depend on the degree to which plantations can be maintained in a state of health. In this chapter, we explore those biotic agents (pathogens, arthropods, and weeds) that are potential constraints to the production of healthy guayule plants. The first three sections of the chapter will treat, in turn, pathogenic and beneficial microorganisms, arthropods, then weeds. Portions of the literature concerning arthropod pests and guayule diseases have been reviewed by several authors (1-3). The detailed review of published research in each section is intended to serve as a compilation of the most pertinent work in the area of guayule health. Within each section, those biotic constraints of greatest potential importance to guayule culture will be highlighted, and specific research needs will be outlined. The fourth section of the chapter will explore the role of toxic substances from *Parthenium* species in terms of allelopathy, allergenicity, and as antimicrobial agents. Finally, the question of a cooperative research strategy for plant health will be addressed with respect to the coordination of efforts among plant breeders, plant pathologists, entomologists, and weed scientists.

PATHOGENIC AND BENEFICIAL MICROORGANISMS

In his early monograph on guayule cultivation, Lloyd (4) describes only two diseases observed on guayule growing in the wild in northern Mexico. The rust disease, caused by *Puccinia parthenii* (Speg.) Arthur, was noted only on plants growing on north-facing slopes of arroyos or in other areas of high relative humidity. The parasitic flowering plant dodder (*Cuscuta* sp. L.) was described as of more importance, with infected plants showing marked dwarfing and stunting, particularly during periods of drought. Since this first description, however, these two organisms have not appeared in any subsequent discussion as important guayule pathogens.

During the Emergency Rubber Project (ERP) period, guayule was propagated almost exclusively in outdoor nurseries, where seedlings were crowded together in beds with frequent overhead irrigation, resulting in fast-growing, succulent plants (5). Since this condition was quite different than the arid environment of its native habitat, it is not surprising that nursery-grown guayule plants were susceptible to many diseases. Among the more important of them were the damping-off diseases associated with the soil-borne fungi *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* sp. (5-8). These diseases were especially favored by cool, moist conditions. Of the damping-off pathogens, *Pythium ultimum* Trow. was predominant in California nurseries (9, 10). On seedlings up to 4 weeks old, tap root lesions were associated with a reddening of cotyledons. On older seedlings, 6 to 16 weeks old, root lesions were accompanied by a pinkish discoloration of the stelar tissues, giving the disease the common name "pink rot." Pathogenicity was demonstrated and the taxonomic characteristics of the fungus elucidated (10). The pathogenicity of *Phytophthora* sp. and *Rhizoctonia* sp. to seedlings was also established in greenhouse tests (8). Seedlings infected with *Rhizoctonia solani* Kuhn had brown to red-brown lesions on the tap root and crown (5). Several fungicides (no longer manufactured), applied as a dry powder to seeds, were found to enhance seed germination and emergence through control of *P. ultimum* and other damping off pathogens (8, 11, 12). *Verticillium* wilt was also considered an important disease of older guayule plants in nurseries (5-7).

The pathogen was originally identified as a microsclerotial-forming strain of *V. albo-atrum* Reinke and Berth., which is now considered to be *V. dahliae* Kleb. The disease was particularly troublesome where nurseries were established on land previously planted to tomatoes or cotton, both highly susceptible to this fungus (5). Cottony rot, caused by the soil-borne fungi *Sclerotinia sclerotiorum* (Lib.) Mass. and *S. minor* Jagger, was also favored by the moist, crowded conditions in nurseries (5-7), particularly near Salinas, California. During August 1942, *Botrytis cinerea* Pers. ex Nocca and Balb. was observed on the older leaves of plants severely damaged by cottony rot (5, 6). Among the fungi noted of minor importance in nurseries was *Sclerotium bataticola* Taub. (= *Macrophomina phaseolina* (Tassi) Goid.) (5).

Several of the diseases of importance in guayule nurseries were also significant in field plantings, particularly since symptomless, infected plants could be inadvertently transplanted into the field, where the disease would continue to develop. One of the most important of such diseases was cottony rot (*S. sclerotiorum* and *S. minor*) (5-7). As in the nursery, the disease was favored by cool, excessively moist conditions (5, 13). Where infected transplanting material was used, death rapidly followed transplanting (14). In surveys of affected fields, *S. minor* was found to be the more prevalent of the two species (13, 14). Campbell (13) has detailed the infection process and the importance of soil moisture to disease development. Although Campbell (15) also described a fungal hyperparasite (*Coniothyrium minitans* Campbell) of the cottony rot pathogens, it was not recommended as a potential biological control agent for these organisms.

Verticillium wilt was another significant disease of field plants during this period, affecting plants of any age (5, 6). The disease was characterized by a blue-grey color change in younger leaves, marginal necrosis of older leaves, and a dark discoloration of stem and root vascular tissues (6). Although *Verticillium* wilt was primarily a problem in the cooler areas of California, it was also observed in Arizona, New Mexico, and Texas (7). Schneider (16-18) examined the susceptibility of several guayule cultivars to *V. dahliae* as well as the role of temperature and soil moisture in disease development. Cultivar 109 was found to be the most severely

affected, while cultivars 405, 407, and 416 were most tolerant, and cultivars 130, 406, and 593 were intermediate in symptom expression. The disease was exacerbated by frequent irrigations and cool temperatures and resulted in a decrease in plant biomass though the percent rubber was unaffected by infection. Gerstel (19) noted a possible relationship between the genetic condition of a cultivar and susceptibility to *Verticillium* wilt, with diploid plants highly susceptible and tetraploid plants significantly more tolerant.

A crown rot attributed to the soilborne fungus *Phytophthora drechsleri* Tucker was reported from California, Arizona, New Mexico, and Texas (5-7, 20). The disease was primarily a problem on heavy, poorly drained soils and was characterized by black, sunken, firm lesions, which would eventually girdle the tap root resulting in plant death (20). It is unclear from the literature whether the pathogenicity of this organism was conclusively demonstrated.

Phymatotrichum root rot of guayule was first noted on eight- to nine-month-old plants growing in Texas in 1930 (21). By October 1930 mortality in the affected field was 6 percent; subsequent inoculation tests confirmed the pathogenicity of the causal fungus *Phymatotrichum omnivorum* (Shear) Duggar. This disease was noted to cause scattered losses of 5 percent or less in fields in Arizona, New Mexico, and Texas (22-24). Where temperatures were favorable for disease development, higher mortality was noted in irrigated rather than in dryland fields (22). Charcoal rot was another root disease of guayule recorded primarily from Texas (25-27). Although the pathogenicity of the soilborne fungus *Macrophomina phaseolina* was not confirmed, it was consistently associated with dark-brown, sunken lesions at the soil line which would eventually girdle the stem, killing the plant. The disease was favored by hot, dry conditions, and Norton (25) suggested disease control through careful irrigation management.

Considered of minor importance during the ERP were a *Fusarium* wilt disease of nursery- and field-grown guayule in Texas, caused by *F. solani* (Mart.) Sacc. (28), and a collar rot associated with a *Phoma* sp. (5). The latter was primarily associated with cool, moist conditions.

The only bacterial pathogen of guayule that has been described caused a root disease of plants in irrigated fields in California (29). The disease was characterized by sudden wilting and the presence of a brown, resinous exudate on stems and roots. Affected cortical tissues turned pink or red, then brown, when exposed to air. The pathogenicity of the bacterium *Erwinia carotovora* f.sp. *parthenii* Starr (30) (= *E. chrysanthemi* Burkholder, McFadden, and Dimock) was confirmed in inoculation tests, and the presence of excess soil moisture was found to be critical for disease development.

One important group of soilborne pathogens that apparently cause virtually no noticeable damage to guayule is the plant parasitic nematodes. A light infestation of root knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood (= *Heterodera marioni* (Cornu) Goodey) was reported from nurseries in California (5). Subsequent greenhouse tests suggest that guayule is actually quite resistant to attack by this parasite (31, 32).

In contrast to the variety and severity of diseases caused by soilborne agents, there are only two reports of pathogens affecting only stems and leaves of guayule. White (33) described a leaf spot disease of guayule in Australia caused by a *Ramularia* sp. As a result of extensive defoliation, affected plants were often stunted, though few were killed. The use of calcium hypochlorite-treated seeds alleviated the problem. A disease of small branches and leaves caused by *Diplodia theobromae* (Pat.) Nowell (= *D. natalensis* P. Evans) was described in Texas (34). Older plants with some dead leaves were more affected than young, succulent plants, leading the author to suggest that the pathogen benefited from a period of saprophytic growth on the dead leaves. Where plants were not crowded, the disease had little effect.

Finally, in experimental inoculations, tobacco mosaic virus and tobacco ring spot virus were able to cause small lesions on guayule leaves (35). It is of interest to note the complete absence of any records of virus diseases in the field during the ERP period, despite the presence of numerous insect vectors.

Seedling damping-off is among the disease problems described during the ERP period that continue to plague guayule culture. Naqvi and Hanson (36) allude to damping-off organisms

affecting seedlings in the greenhouse, but it is not clear whether, in fact, they actually isolated and identified the fungi that were discussed. Several new *Pythium* species have been confirmed as seedling pathogens. *P. aphanidermatum* (Edson) Fitzpatrick was found to rot 2- to 24-month-old seedlings in Arizona, primarily between June and October (37). In several cases both *P. aphanidermatum* and *Macrophomina phaseolina* were recovered from the same diseased plant. *P. dissotocum* Drechsler and *P. paroecandrum* Drechsler were both confirmed as damping-off pathogens of 2- to 6-week-old seedlings in Arizona (38). In studies of strategies for the management of damping-off, the use of the combination of the solar blanket material, Reemay, and certain fungicides, was found to significantly enhance germination of directly sown guayule seeds and seedling establishment in the field (38). In greenhouse tests to control damping-off, various fungicides were incorporated into a magnesium silicate fluid-drilling gel that contained pregerminated guayule seeds (39). PCNB (pentachloronitrobenzene) was effective in controlling *R. solani*, while Captan (N-[Trichloromethylthio] -4-cyclohexene-1, 2-dicarboximide) and Fenaminosulf (Sodium (4-[dimethylamino] phenyl) diazene sulfonate) were effective in controlling *Pythium debaryanum* Hesse.

Recent work with *Fusarium* spp. as seedling pathogens has again been contradictory with respect to the pathogenicity of particular species. In a series of growth-chamber studies, Ykema and Stutz (40) established the pathogenicity of two isolates of *F. oxysporum* Schlect. em. Sny. & Hans. However, they were unable to confirm the pathogenicities of *F. solani* and *F. roseum* Schwabe isolates recovered from diseased guayule roots. A recent study in India confirmed the role of *F. solani* as a seedling pathogen during the rainy season (41).

Phytophthora cryptogea Pethybridge and Lafferty was confirmed as a seedling pathogen in California (42). The fungus caused 100 percent mortality in one experimental plot, and the authors noted that diploid seedlings were markedly more susceptible than polyploids. *Rhizoctonia solani* was reportedly associated with transplant mortality in Gujarat State, India, where poor drainage was a problem (43). Recently, it has been determined that binucleate *Rhizoctonia* isolates, which were morphologically similar to the multinucleate *R. solani*, were

nonpathogenic to guayule seedlings, emphasizing the need for characterization of the nuclear condition of *Rhizoctonia* isolates as a part of the diagnostic process (44).

The charcoal rot pathogen *Macrophomina phaseolina* has been recorded from dying plants up to four years old in the field in Arizona (45). In tests of 15 triploid and tetraploid cultivars, no disease resistance and no differences in cultivar susceptibility were found. Greenhouse experiments provided preliminary evidence that saline irrigation water enhanced disease development. Another root disease observed in one field in Arizona was *Phymatotrichum* root rot (Mihail and Alcorn, 1984, unpublished).

Several recent studies have focused on evaluating cultivar tolerance to the *Verticillium* wilt pathogen. In greenhouse tests with USDA tetraploid and triploid cultivars, the most tolerant cultivars were: 593, 11605, 12229, AZ101, 11693, 4265XF, A48118, N576, while those least tolerant were 12231, N396, 11633, N575, and N565 (46). Cheo and Beaupre (47) confirmed the observations of Gerstel (19) that diploid plants are more susceptible to *Verticillium* wilt than are polyploids. In general these authors noted that other *Parthenium* spp. were much more tolerant of *Verticillium* wilt when compared to *P. argentatum*. Finally, in a growth chamber study, Stutz (48) found that 1-week-old seedlings were more susceptible than 10-week-old plants to *V. dahliae* infection.

In recent experimental plantings in New South Wales, Australia, root diseases associated with heavy, poorly drained soils have been observed (49, 50). The fungi isolated which were most likely to be pathogens were *Phytophthora drechsleri*, *Fusarium solani*, and *Rhizoctonia* sp.

In a greenhouse test involving the plant parasitic nematodes *Helicotylenchus pseudorobustus* (Steiner) Golden, *Meloidogyne incognita* race 3, *Pratylenchus scribneri* Steiner, and *Criconemella xenoplex* (Raski) Luc. and Raski, Thomas (51, 52) found that none affected guayule growth. Only the population of *C. xenoplex* increased in the presence of guayule, while the populations of the other three species decreased or were eliminated. These studies confirmed the conclusions of Campbell (5) and Hoyman (31, 32) that guayule would be largely unaffected by nematodes.

Finally, a malady of unknown cause has been noted in accession 79-031 growing at Pecos, Texas (53). The syndrome is characterized by a witches' broom, leaf distortion, prolific blooming, and poor filling of seeds.

The potential of the various described pathogens to constrain guayule production will depend in large measure on the methods selected to establish plantations, cultural procedures, and on the climatic conditions of the production areas. If fields are established from transplants grown in field nurseries or in greenhouses where cultural practices are poor, many of the diseases described during the ERP will likely reappear. However, so long as guayule research and cultivation are focused in the hot arid areas of the world, it is unlikely that cottony rot (*Sclerotinia* spp.) or *Verticillium* wilt will be as damaging as reported during the ERP period. The diseases of concern for established plantings will probably be those favored by hot and/or dry conditions such as charcoal rot and *Phymatotrichum* root rot.

The most pressing need for future pathology research is to develop management strategies for the control of diseases associated with directly sown guayule seeds, very young seedlings, and new transplants. Control of diseases of established plants (e.g., charcoal rot, crown rot) cannot cost-effectively depend on chemical protectants. Rather, if sustained funding is available, three other approaches appear to have the most promise at this time. These are: 1) for the immediate future, the initiation of a comprehensive breeding program that includes disease resistance as a high priority (54); 2) basic investigations focused on the development of biological control protocols; and 3) basic research to develop transgenic plants containing resistance genes. As a reminder, however, quality disease control starts with quality farm management practices.

Not all microorganisms associated with guayule are detrimental. In recent studies Bloss and co-workers have identified several species of the endosymbiotic vesicular-arbuscular mycorrhizal (VAM) fungi associated with the roots of native and cultivated guayule (55-59). Both *Glomus deserticola* Trappe, Bloss & Menge (59) and *G. fasciculatum* (Thaxter) Gerd. &

Trappe (55) were observed to naturally form symbiotic associations with guayule roots in the southwestern United States. In greenhouse and field studies comparing guayule inoculated with VAM fungi (*G. intraradicies* Shenk & Smith and *G. fasciculatum* biotype A) with uninoculated plants, biomass, nutrient content (Ca, Mg, Zn), and latex accumulation were found to be higher in the VAM-inoculated plants (56-58). Additionally, VAM inoculation of transplants greatly enhanced survival in the field. Further work (60-62) suggests that VAM-inoculated guayule might be better able to withstand highly saline conditions than uninoculated plants. In greenhouse work with growth-promoting rhizobacteria *Pseudomonas fluorescens* (Flugge) Migula dramatic growth increases of inoculated guayule were observed, leading the authors to suggest a role for these bacteria in the production of transplants (63). Future research in guayule culture must not overlook the importance of such beneficial micro-organisms. As new areas are brought into guayule cultivation, it will be important to ascertain the presence of a resident VAM fungal population. If these fungi are lacking, methods for their introduction will need to be developed.

ARTHROPOD PESTS

Major arthropod pests of guayule are listed in Appendix 1. The earliest known report of such pests is that of Lloyd (4) describing insects observed on native and cultivated guayule in Mexico. Three scale insects (*Ceroputo yuccae*, *Orthezia* sp., and *Targionia dearnessi*) were noted feeding on roots. The bark beetle *Pityophthorus nigricans* was observed tunneling in harvested shrub awaiting processing, leading to the recognition that harvested guayule must be processed expeditiously.

Of all the guayule insect pests described during the ERP, the plant bugs (*Lygus* spp.) probably received the most attention (64, 65). *L. hesperus* was the predominant species, with *L. nigritus*, *L. apicalis*, *L. sallei*, and *L. elisus* also noted. Under the irrigated conditions in

California, guayule flowered continuously from spring to fall. These *Lygus* spp. caused serious damage through feeding on the developing seeds and succulent growing tips (65). Further investigations (66) suggested that these insects injected a toxin into the host, resulting in localized cell death. Romney and Cassidy (67) also noted that the wasp *Anaphes ovijentatus* was parasitic on the eggs of these species. Another member of the Miridae, *Sixeonotus areolatus*, caused damage in Texas (68).

Several arthropod pests of greenhouse-grown guayule were described during the ERP period. Two mites, *Aceria parthenii* and *Tetranychus bimaculatus*, caused leaf distortion, particularly in warm conditions (68, 69). Cassidy et al. (68) further noted seedling damage by crickets (*Acheta* sp.), aphids (*Myzus persicae*, *Aphis gossypii*), mealybugs (*Phenacoccus gossypii*), whiteflies (*Trialeurodes vaporariorum*, *Aleyrodes spiraeoides*), leafhoppers (*Empoasca arida*), and leaf miners (*Phytomyza atricornis*).

Lange (70) and Cassidy et al. (68) surveyed insect pests of cultivated guayule in nurseries and fields in California and the southwestern United States. Thrips (*Frankliniella occidentalis*, *F. occidentalis trehernei*, *F. moultoni*, *F. minuta*, *Thrips tabaci*, *Chirothrips aculeatus*, and *Aelothrips* sp.) were found in large numbers on small plants, causing leaf curling and distortion. Near Oceanside, California, a darkling ground beetle, *Ulus crassus*, killed about four plants per square foot in a nursery by feeding on shoot-tips. Aphids were occasionally abundant in California nurseries but were usually controlled by natural predators. Among the reported aphids were: *Myzus persicae*, *Aphis gossypii*, and *Anuraphis helichrysi*. Wireworms were found to be widespread in California nurseries and particularly damaging to the roots of small seedlings. Three wireworm species noted were *Limonius californicus*, *L. canus*, and *L. infuscatus*. Lange (70) reported the identification of 14 species of leafhoppers of which *Empoasca arida*, *Macrosteles divisus*, *Draeculacephala minerva*, and *Carneocephala fulgida* were the most common. Despite the frequency of occurrence, little damage was attributable to leafhoppers. However, the woolly-bear caterpillar *Estigmene acraea* skeletonized leaves while the guayule stem borer

Agromyza virens caused extensive damage to stems as a result of larval feeding. Lange also noted the presence of two chalcid parasites, *Syntomopus americanus* and *Halticoptera aenea*, which kept borer populations in check. Ants (*Pogonomyrmex* spp.) were found to glean seed and remove the cotyledons of small seedlings in a nursery near Indio, California. Among the other nursery insect pests of less significance, Lange (70) includes June beetles (*Ligyrus californicus*), armyworms (*Laphygma* sp.), zebra caterpillars (*Mamestra picta*), and weevils (*Listroderes obliquus*).

Cassidy et al. (68) described grasshoppers as the most destructive insect pests of field plantings. Heavy grasshopper infestations may result in defoliation, decortication, and eventual death of plants. Among the more common species were: *Melanoplus mexicanus devastator*, *M. cinereus cyanipes*, *M. differentialis*, *M. marginatus*, *M. femur-rubrum*, and *Oedaleonotus enigma*. Cortex-feeding termites, *Amitermes tubiformans*, were observed in Texas. Three species of ants were considered to be serious potential guayule pests (68). Fire ants, *Solenopsis xyloni* var. *maniosa*, were observed to feed on the root cortex in California. The red harvester ant *Pogonomyrmex barbatus* caused defoliation of transplants in circular areas centered on colonies in New Mexico and Texas. The leaf-cutting ant *Atta texana* also caused defoliation of plants adjacent to colonies in Texas. In Texas and New Mexico, damage to foliage and stems was also attributable to lacebugs (*Corythucha morrilli*) and the garden webworm *Loxostege similalis* (68) and in Texas to a "gall fly" (Cynipidae) (70). Lange (70) also noted the wide distribution of the root scale *Rhizaspidiotus dearnessi* which had been earlier recorded by Lloyd (4) as *Targionia dearnessi*. Specimens identified as carrot beetles, June beetles, *Ligyrus gibbosus*, and/or *L. californicus* caused damage to transplants in Arizona, California, and Texas (23, 68, 70). In an Arizona field planting established to examine guayule diseases, more than 75 percent of the 10,000 transplants were killed by the feeding of June beetle larvae, and possibly adults (23). Variable damage to field plantings was also caused by aphids, leafhoppers, wireworms (*Limonius* spp.; *Melanotus* sp.), and the salt-marsh caterpillar (*Estigmene* acera) (68).

Romney (71) reported several new insects from a survey of native and cultivated guayule in northern Mexico in addition to the guayule borer of Lloyd (4). Two root scales (*Targionia* sp. and *T. yuccarum*) caused stunting of native plants. Two branch scales (*Lecaniodiaspis* sp. and *Tachardiella cornuta*) were also described from native guayule. Larvae of several beetles (*Urilleta* sp., *Chalcophora* sp., and *Dicerca* sp.) were noted on harvested shrub awaiting processing. Larvae of *Eucosma* sp. were observed boring on roots of native shrubs. Among the other insects common on cultivated guayule were: grasshoppers (*Encoptolophus pallidus*, *Platylactisca azteca*, and *Trachyrhachys kiowa*) and the plant bug *Polymerus basilis*. The leafhopper *Cloanthanus heldoranus* was common on native and cultivated plants, while *Empoasca* spp. were common only on cultivated guayule.

Several published reports during the ERP period dealt with controlling the insect pests of guayule (68, 72-74). Most of the research confirmed the efficacy of DDT and various arsenicals for control of these pests. Since the use of these compounds is currently prohibited, present insect management strategies will require new approaches.

During the ERP, many guayule plantations were established in sites (e.g., coastal California) that are not typical of current arid guayule-growing areas. Thus, insect pests that were important in California would probably not be of primary importance under nonirrigated, arid-land cultivation. Those pests that were most severe in fields in Arizona, New Mexico, and Texas (grasshoppers, ants, and lace bugs) are probably typical of those that will continue to constrain guayule production.

Since the ERP, there have been only a few intensive investigations of arthropods associated with guayule (75-78). In these studies, extensive collections were made in the field, and all identified insects are listed along with comments on abundance. While the highlights of these surveys are presented here, the original sources should be consulted for details. A survey of native and cultivated guayule growing in two localities in northern Mexico yielded 290 arthropod species distributed among nine orders (76, 77). Several types of damage to guayule plants were noted, including: defoliation (Lepidoptera: Nymphalidae, Coleoptera: Curcu-

lionidae, Orthoptera: Acrididae), leaf perforation (*Bucculatrix* spp.), damage to seeds and buds (*Smicronyx* spp., *Lygus* spp.), sucking injury to leaves and stems (*Empoasca* spp. and other specimens from the Homoptera families, Acanaloniidae and Flatidae), and boring damage to roots and branches (*Pityophthorus* sp.). The second survey of native and cultivated guayule in northern Mexico (75) revealed the occurrence of 97 species of entomophagous insects in five orders and 37 families that might be beneficial in controlling guayule insect pests. Among the Coleoptera, the most frequently represented families and species were: Anthicidae (*Anthicus* spp., *Notoxus* spp.), Malachiidae (*Collops* spp.), and Coccinellidae (*Hippodamia convergens*, *Symnus* sp.). Among the Hemiptera, the most frequently recorded families and species were: Anthocoridae (*Orius tristicolor*), Lygaeidae (*Geocoris* spp.), and Reduviidae (*Zelus renardii*). Representatives of the Diptera, Hymenoptera, and Neuroptera were also observed.

In a survey of guayule growing areas in west Texas from 1979 through 1983, 107 arthropod species from 13 orders and 60 families were found (78). Taxa of potential importance, owing largely to their frequency, were: the Morrill lace bug *Corythucha morrilli* and other lace bugs, and *Xyonysius californicus*, *Nysius* spp., *Lygus* spp., and *Empoasca* spp. (78). *Acanthoscelides pallidipennis*, though not common, was considered to be potentially important, since it breeds in the seeds of other plant species, thus potentially restricting guayule seed production. Among the other commonly collected insects were the green peach aphid (*Myzus persicae*), spotted cucumber beetle (*Diabrotica undecimpunctata howardi*), tobacco flea beetle (*Epitrix hirtipennis*), and several ants. During one season, in one field, the Banks grass mite (*Oligonychus pratensis*), was observed to cause significant damage. Among the possible beneficial insects recorded, three species were particularly common: *Orius tristicolor*, *Nabis alternatus*, and *Geocoris pallens*. In further work with the Morrill lace bug, Stone and Watterson (79) noted extensive damage to plants both in greenhouses and in the field in Texas and described the effects of temperature on insect development.

The potential importance of the lace bug as a guayule pest (78) was further emphasized by observations of guayule defoliation by *Corythuca ciliata* in a field planting in southern Califor-

nia (42). The authors noted successful control of the pest with Orthene. In 1961 two weevil species, *Microlarinus lareynii* and *M. lypriformis*, were introduced into the United States from India to control puncture vine weed (*Tribulus terrestris* L.). During the fall of 1977, both species were observed to feed on and damage guayule leaves and green stems in a field planting (42). The authors reported that Malathion gave effective control. Elsewhere, *Lygus* spp. (especially *L. hesperus*) and the associated egg parasite *Anaphes ovijentatus* were noted in an experimental guayule field in Tucson, Arizona (80). Milthorpe (49) observed aphids, Rutherglen bugs, cutworms, and *Heliothis* spp. caterpillars in experimental guayule plantings in New South Wales, Australia, though only minor damage was inflicted. Thrips (*Microcephalothrips abdominalis*) have been observed on guayule in Lucknow, India, and a role in pollination has been suggested (81). Larvae of *Dasychira mendosa* were observed feeding on guayule leaves in Tamil Nadu, India (82). It has recently been demonstrated that the sweet potato whitefly (*Bemisia tabaci*) can complete its life cycle on guayule under experimental conditions (83). Finally, there has been a recent report that guayule plants with high rubber content have fewer insect pests, an observation that the authors suggest might be turned to advantage in the selection of high-yielding plants (84).

Both ERP and recent observations suggest that aphids and leafhoppers are common on guayule though they rarely cause damage. On the other hand, these insects, plus whiteflies, are important vectors of plant-pathogenic viruses. Although virus diseases of guayule are not yet recognized in the field, the presence of known vectors suggests that the potential for the occurrence of virus diseases, and their possible constraints to guayule production should not be underestimated.

Since the ERP, most reports of insects associated with guayule have been based on field surveys. The most extensive observations of arthropod damage to guayule in the greenhouse appear to be those of Naqvi and Hanson (36). Over a three-year period those arthropods that were most common were: aphids (*Rhopalsiphum nymphaeal*, *Pentalonia nigronerosa*, *Macrosyphum* spp.), loopers (*Trichoplusia ni*, *Hyphentria cunes*, *Platynota stultana*), mealy-

bugs (*Pseudococcus* spp.), whiteflies (*Trioleurodes vaporariosum*), thrips (*M. abdominalis*), and mites (*T. urticae, T. witical*).

The recent survey in Texas (78) has probably recorded most insect genera likely to be constraints to guayule cultivation in the southwestern United States and northern Mexico. However, as new arid areas are brought into guayule production around the world, it is likely that additional arthropod species will be identified. During the ERP period, many arthropods were described as problems in greenhouses and nurseries. As the difficulties associated with plant establishment in the field from directly sown seed are resolved, many of these arthropods could cease to be of major concern. However, a shift to direct seeding will probably increase problems caused by ants gleaning seed and directly damaging the young seedlings. Alleviation of arthropod predations in established field plantings will require the judicious integration of new techniques with cultural and chemical strategies, the guidelines for which may well have to be developed on a local basis. Important considerations are the possibilities of biological control (36) and the breeding for pest resistance (85).

WEEDS

Weed control in guayule nurseries was one of the most expensive operations in the production of guayule rubber by the ERP in 1942. Approximately 3,000 people were used to hand weed 223 ha of nurseries near Salinas, California. This labor requirement was reduced 90 percent by the use of close cultivation between the bands of plants and through the development of post-planting petroleum oils (86). Again, in 1951 oil sprays gave effective control of seedling grasses and some broad-leaved weeds on 472 ha of guayule plants being raised for the Guayule Stockpiling Project near Crystal City, Texas (3, 87, 88). As selective weed oils have a limited weed spectrum and are no longer cost effective, research in the 1970s and 1980s has focused on the evaluation of modern herbicides (Table 1). From 1978 to 1981 ten herbicides were tested in New Mexico on directly seeded guayule and 15 herbicides on transplanted guayule (87, 89, 90).

Table 1. A summary of herbicides cited in the text that have been experimentally used with guayule.[a]

Common name	Chemical name[b]
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
Chlorsulfuron	2-chloro-N-[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl) amino] carbonyl] benzenesulfonamide
DCPA	dimethyl 2, 3, 5, 6-tetrachloro-1, 4-benzenedicarboxylate
Diuron	N'-(3, 4-dichlorophenyl)-N, N-dimethylurea
Glyphosate	N-(phosphonomethyl) glycine
Metribuzin	4-amino-6-(1, 1-dimethylethyl)-3-(methylthio)-1, 2, 4-triazin-5(4H)-one
Oryzalin	4-(dipropylamino)-3, 5-dinitrobenzenesulfonamide
Oxyfluorfen	2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene
Paraquat	1, 1'-dimethyl-4, 4'-bipyridinium ion
Pendimethalin	N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenamine
Prometryn	N, N'-bis (1-methylethyl)-6-(methylthio)-1, 3, 5-triazine-2, 4-diamine
Simazine	6-chloro-N, N'-diethyl-1, 3, 5-triazine-2, 4-diamine
Trifluralin	2, 6-dinitro-N, N-dipropyl-4-(trifluoromethyl) benzenamine
2, 4-D	(2, 4-dichlorophenoxy) acetic acid
Varsol Naptha™ Oil	

[a] As of 1/4/89 none of these chemicals have been registered for use with guayule.
[b] Chemical names are from the listing in *Weed Science* 36(6), 1988.

Some of these same herbicides were evaluated for weed control in transplanted guayule in California (91) and in Arizona (92). Eighteen herbicides were also tested in Australia as preplant treatments for control of weeds that would compete with seedling guayule and also as treatments for weeds among established guayule (50).

Direct field seeding produces guayule seedlings that are slow growing and that offer little competition to the seedlings of weeds emerging at the same time. An effective preplant or preemergence herbicide is essential for the success of this method of guayule establishment. In the search for such a herbicide, 13 chemicals or chemical combinations were evaluated in greenhouse experiments (93, 94). Of the 13, only DCPA and pendimethalin demonstrated adequate selectivity on directly seeded guayule. Additional experiments carried out under field conditions confirmed the results of the greenhouse tests (95). Applied as preplant, soil-incorporated treatments DCPA at 8.98 kg/ha and pendimethalin at 1.12 kg/ha reduced the stands of guayule 5 percent and 22 percent, respectively. Additional field tests at other locations in New Mexico further established the reported difference between these two herbicides. Both gave an average control of 94 percent of the *Amaranthus* L. weed species and were very effective in preventing establishment of seedling Gramineae. However, when compared on 12 other weed species, DCPA gave only 60 percent control and pendimethalin 68 percent. Thus, there is still a need for a herbicide but with a broader spectrum of weed control.

No modern postemergence herbicide has been tested that will control weeds in directly seeded fields when the guayule plants are still in the seedling stage. Varsol Naptha™ oil sprays used in 1951 on 472 ha of guayule nurseries were very effective on small seedling grasses and on some types of broad-leaved weeds, but when the weeds were of the *Asteraceae*, *Cruciferae*, *Malvaceae*, or *Umbelliferae* families they were not controlled (3, 96, J.W. Whitworth, unpublished).

In transplanted guayule, weed control is less of a problem than in fields established from directly sown seed. The older and larger guayule plants are not only more competitive with weeds, but are also more tolerant of various herbicides than are young seedlings. Greenhouse

and field experiments begun in New Mexico in 1978 indicate that guayule transplanted into soils treated with herbicides of the dinitroaniline group (trifluralin, pendimethalin, and oryzalin) sustained no injury that would cause stand or growth reduction (93, 96). Members of this group of herbicides are very effective in preventing the establishment of many summer annual weeds, but are ineffective on many winter annual weeds, as well as those not controlled by weed oils. Broader spectrum herbicides (e.g., the triazine herbicide, simazine, and the substituted urea herbicide, diuron) caused reductions in stands and growth of transplanted guayule in New Mexico and California (87, 89, 91-93). Clark found that two other triazines (metribuzin and prometryn) were also highly phytotoxic to guayule (87). However, on different soils and under drought conditions in Australia, guayule stands and growth on plots treated with triazine and substituted urea herbicides sometimes exceeded that on the untreated check plots (50). This anomaly was undoubtedly due to lack of sufficient water to move the herbicides into the root zone of the guayule and to the excellent control of shallow emerging weeds, thereby reducing the severe competition by the dense stand of weeds that emerged on the untreated check plots. Whitworth demonstrated that simazine was too hazardous to use on sandy soils of different pH taken from each of three different locations in Australia (49). In this glasshouse experiment, severe stand and growth reductions of guayule occurred with all three rates of simazine when incorporated into the soil around the roots of four-month-old guayule transplants. Differences in soil pH were without apparent influence on the phytotoxicity of simazine to the guayule transplants. The phytotoxicity of trifluralin was slightly greater on neutral and alkaline soils than on the acidic soil. In the same experiment, oxyfluorfen proved safe for guayule on all three soils. When used in combination with trifluralin, the combination performed similarly to trifluralin alone. Earlier experiments in California (91) and New Mexico (90, 94) had established that oxyfluorfen was safe to use on transplanted guayule, but greenhouse tests in New Mexico showed that it was too toxic to use on directly seeded guayule seedlings or as either a preplant or preemergence herbicide. Oxyfluorfen is effective on winter annual weeds that are not controlled by the dinitroanlines, such as trifluralin. In Arizona

serious weed problems include winter annual weeds, such as: London rocket (*Sisymbrium irio* L.), little mallow (*Malva parviflora* L.), prickly lettuce (*Lactuca serriola* L.), spiny sowthistle (*Sonchus asper* (L.) Hill), and wild turnip (*Brassica tournefortii* Gouan.) in established guayule plantings (2).

Combining such herbicides as oxyfluorfen with oryzalin or trifluralin can increase the spectrum of weeds controlled. A tank mix of oxyfluorfen plus oryzalin at a rate of 2+2 kg/ha applied on the surface of the soil controlled weed growth in young, transplanted guayule for a period of four to six months in Australia (49, 50, 88, 97). The untreated areas of the .75-ha field became heavily infested with common heliotrope (*Heliotrophium europaeum* L.) and with scatterings of other weeds, including such perennials as *Solanum elaegnifolium* Cav. and *Chondrilla juncea* L. The emergence of the perennial weeds was severely retarded by the treatment, but these weeds were not killed and were removed by hoeing or by spot treatment with glyphosate. Had this herbicide mixture been available in 1981, weed control on 12 ha of guayule for seed increase in New Mexico would have been much more effective than the trifluralin treatment that was used (J.W. Whitworth, unpublished).

Weed control in older, established fields of guayule can be a problem since the size of the guayule plants may prohibit mechanical cultivation. In west Texas, severe infestations by both warm- and cool-season weeds occurred in a four-year-old stand of guayule (98, 99). Rocket mustard (*S. irio*) was the dominant weed species. Over 90 percent control of the broad-leaved weeds was accomplished by applications of broadcast sprays of glyphosate at 1.1 kg/ha or bromoxynil or 2,4-D at 0.8 kg/ha during the dormant season for guayule. Injury to the guayule during the growing season was avoided by a rope-wick application of glyphosate to the weeds extending above the shrub canopy. The primary invading weed species on the 12 ha, seed-increase block of guayule in New Mexico was Russian thistle (*Salsola iberica* Sennen and Pau). Cleanup operations have been required in each of the years since the establishment of this field.

Rubber production may be influenced by the application of recommended herbicides. This problem has received some attention by Clark (87), Elder (91), and Garcia-Avila (100). General information to date indicates that unless the herbicide treatment reduces the stand of guayule, rubber yields are not reduced (87, 89, 91, 100, 101). However, workers have noted a decrease in resin production due to herbicide treatments (89, 100).

A differential response to herbicides by guayule cultivars has been observed. Clark and Whitworth (89) reported that a treatment of granular simazine reduced the stand of guayule cultivar 4265XF by 29 percent versus no stand reduction for cultivar 11619. On sandier soils with less clay and organic matter content, a 100 percent kill of stands of both cultivars would be expected.

Problems associated with the use of herbicides include obtaining clearance from the U.S. Environmental Protection Agency for their use in guayule fields. Although no crop residue limits must be established for this nonfood crop, efficacy data are required. A company must then determine that the use of the herbicide on guayule will guarantee a profit in excess of labeling costs and of crop-damage suits. Another problem, the high costs of some of these herbicides, can be partially solved by narrow-band applications along the row instead of broadcast applications over the top, thereby reducing the amount of herbicide required by one-third. Using this approach, Stewart and co-workers (88) have put together a program for weed control in a 10-year-old stand of guayule established by seed, ratooned after 5 years, and dug at the end of 10 years. Basic herbicides included in this program are DCPA at seeding time and paraquat and glyphosate as shielded, band sprays from the second year on. Surviving weeds would be removed by cultivation between the rows and by hand weeding in the rows. Cost estimates are included for this program.

Future weed-control research should focus on all new herbicides in a search for a broad-spectrum chemical that will safely control weeds in fields of directly seeded, newly transplanted, and/or well-established guayule. Chemical companies are currently investigating new groups of herbicides that require application rates 1/50th that of simazine for effective weed

control. One of these, chlorsulfuron, at 0.04 kg/ha showed limited selectivity on guayule (49). Safer compounds for use on guayule may be found in other derivatives of this or other new groups of herbicides. Ultimately, before any herbicide is recommended for weed control in guayule, its influence on the dry matter, resin, and rubber production of the candidate guayule cultivars must be thoroughly investigated.

PARTHENIUM *PRODUCTS AS TOXINS*

The previous sections of this chapter have focused on organisms as constraints to guayule production. It is time to consider the benefits and problems associated with those *Parthenium* constituents that are allelopathic or toxic to other organisms, including man. In 1945, Bonner and Galston (102) observed that in dense nursery plantings of guayule seedlings, those plants in the edge rows were larger than those in the center. As a result of studies with laboratory-grown seedlings, they identified a group of allelopathic compounds, including trans-cinnamic acid. However, further work (103) failed to establish the accumulation of these compounds in soil under field conditions.

More recently, Isman and Rodriguez (104, 105) demonstrated that the sesquiterpene lactones present in leaves of some *Parthenium* spp. (not *P. argentatum*) are effective larval growth inhibitors of two common plant pests, bollworms (*Heliothis zea* Boddie) and beet armyworms (*Spodoptera exiqua* Hübner). The authors postulated that these compounds serve *Parthenium* spp. as effective natural defenses against phytophagous insects. Of the three species that produce rubber, *P. incanum* H.B.K. and *P. integrifolium* L. also produce sesquiterpene lactones (105). Mears and Larson (106) caution that hybridization of the latter two *Parthenium* species with *P. argentatum* may result in the unintentional introduction of the genes for these potentially allergenic lactones into resultant hybrids. Guayule also produces its own contact allergin, a sesquiterpene cinnamic acid ester, guayulin A (107). Although these reports indicate a potential problem for guayule development, it should be possible to integrate safety precautions

into the harvest and extraction processes to minimize prolonged dermal contact or the inhaling of particulates.

The occurrence of such toxic *Parthenium* products is not entirely negative. Bultman and co-workers (108) found that pine sapwood impregnated with guayule resin was nearly impervious to attack by molluscan wood borers (*Limnoria tripunctata* Menzies and *Martesia* sp.) when treated wood was submerged in the Panama Canal. Similarly, terrestrial termites of the genera *Coptotermes* and *Heterotermes* (Isoptera: Rhinotermitidae) avoided feeding on resin-impregnated pine sapwood (108) and on resin-impregnated polyvinyl chloride electrical insulation (109). Further studies (110, 111) demonstrated inhibition of wood decay by selected brown rot fungi (*Gloeophyllum trabeum* [Pers. ex Fr.] Murr., *Antrodia carbonica* [L.O. Overholts] L. Ryvarden and R.L. Gilbertson, *Fomitopsis cajandri* [Karst.] Kotl. et Pouz., and *Lentinus ponderosus* O.K. Miller) and white rot fungi (*Dichomitus squalens* [Karst.] D. Reid, *Trametes versicolor* [L. ex. Fr.] Pilát, and *Ganoderma* sp.) which were growing on resin-impregnated pine sapwood. Similarly, soft-rotting fungi growing on resin-impregnated birch or pine sapwood failed to cause decay. The results of these studies strongly suggest a large potential for guayule resins in the preservation of "in-service" lumber. Indeed, guayule resin may prove to be as valuable a product as the rubber, which was the first focus of interest.

SUMMARY AND FUTURE RESEARCH

Although a number of biotic agents have been described during the past century as potentially constraining to guayule production, still, the plant is host to far fewer pathogens and arthropod pests than many other agronomic and horticultural crops. In short, guayule is a remarkably hardy plant.

As guayule is cultivated in new areas, undoubtedly, the list of associated diseases, arthropods, and weeds will grow. Therefore, the general approach to the maintenance of plant health

should reflect the multidisciplinary nature of the problems. The use of protectant and therapeutic chemicals, particularly for disease control, should be considered interim measures. Only cooperative plant breeding programs including breeders, pathologists, and entomologists will result in new cultivars that combine both high rubber content and increased tolerance to pathogens and insect pests. Likewise, cooperation among all disciplines concerned with guayule culture should focus on integrated management systems that will incorporate disease, insect, and weed management strategies, thus reducing dependence on chemicals, which will undoubtedly become increasingly costly and difficult to apply as public scrutiny grows.

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Appendix 1. Some arthropods associated with guayule.

Taxonomic grouping	Some areas of occurrence
ARACHNIDA	
Eriophyidae (gall mites)[a]	
Aceria parthenii Keifer	CA
Tetranychidae (spider mites)	
Oligonychus pratensis (Banks)	TX
Tetranychus urticae Koch	CA
[as *Tetranychus bimaculatus* Harvey][b]	
[as *T. Witical*]	
INSECTA	
COLEOPTERA (beetles)	
Anobiidae (anobiid beetles)	
Vrilleta sp.	Mexico
[as *Urilleta* sp.]	
Anthicidae (ant-like flower beetles)	Mexico
Anthicus spp.	Mexico
Notoxus spp.	Mexico
Bruchidae (seed beetles)	
Acanthoscelides pallidipennis (Motschulsky)	TX
Buprestidae (metallic wood-boring beetles)	
Chalcophora sp.	Mexico
Dicerca sp.	Mexico

[a]Scientific names from: Werner, F.G. 1982. *Common names of insects and related organisms, 1982*. Entomological Society of America. 132 pp. Family common names from: Borror, D.J., D.M. DeLong, and C.A. Triplehorn. 1981. *An introduction to the study of insects, fifth edition*. Philadelphia, Saunders College. 928 pp.

[b]Original citations are in brackets when they differ from current spellings and/or classification.

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
COLEOPTERA (continued)	
Chrysomelidae (leaf beetles)	
Diabrotica undecimpunctata howardi Barber	CA, TX
Epitrix hirtipennis (Melsheimer)	TX
Coccinellidae (lady beetles)	
Hippodamia convergens Guérin-Méneville	Mexico
Scymnus sp.	Mexico
[as *Symnus* sp.]	
Curculionidae (weevils)	Mexico
Listroderes costirostris obliquus (Klug)	CA
[as *Listroderes obliquus* Gyllenhal]	
Microlarinus lareynii (Jacquelin du Val)	CA
M. lypriformis (Wollaston)	CA
Smicronyx spp.	Mexico
Elateridae (click beetles)	
Limonius californicus (Mannerheim)	CA
L. canus LeConte	CA
L. infuscatus Motschulksky	CA
Limonius spp.	CA
Melanotus sp.	CA
Melyridae (soft winged flower beetles)	
[as **Malachiidae**]	
Collops spp.	Mexico

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
COLEOPTERA (continued)	
Scarabaeidae (scarab beetles)	
Bothynus gibbosus (DeGeer)	AZ, CA, TX
[as *Ligyrus californicus* Casey]	
[as *Ligyrus gibbosus* (DeGeer)]	
Scolytidae (bark beetles)	Mexico
Pityophthorus mexicanus Blackman	Mexico
[as *Pityophthorus nigricans* Bland]	
Pityophthorus sp.	Mexico
Tenebrionidae (Darkling ground beetles)	
Ulus crassus (LeConte)	CA
DIPTERA (flies)	CA, Mexico
Agromyzidae (leafminer flies)	
Melanagromyza virens (Loew)	CA
[as *Agromyza virens* (Loew)]	
Phytomyza atricornis Meigen	CA
HEMIPTERA (bugs)	
Anthocoridae (minute pirate bugs)	
Orius tristicolor (White)	Mexico, TX
[as *Orious tristicolor* White]	
Lygaeidae (seed bugs)	
Geocoris pallens Stal	TX
Geocoris spp.	Mexico

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
HEMIPTERA	
Lygaeidae (continued)	
Nysius vinitor Bergroth [as Rutherglen bug][c]	Australia
Nysius spp.	TX
Xyonysius californicus (Stal)	TX
Miridae (leaf bugs)	
Lygus apicalis Fieber	CA
L. elisus Van Duzee	CA
L. hesperus Knight	AZ, CA
L. nigritus Knight	CA
L. sallei Stal	CA
Lygus spp.	CA
Sixeonotus areolatus Knight	TX
Nabidae (damsel bugs)	
Reduviolus alternatus (Parshley) [as *Nabis alternatus* Parshley]	TX
Reduviidae (assassin bugs)	
Zelus renardii Kolenati	Mexico
Tingidae (lace bugs)	
Corythucha ciliata (Say) [as *Corythuca ciliata*]	CA

[c]Only the common name originally cited.

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
HEMIPTERA	
Tingidae (continued)	
C. morrilli Osborn & Drake	NM, TX
Corythucha spp.	AZ, NM, TX
Family uncertain	
[as *Polymerus basilis* (Reuter)]	Mexico
HOMOPTERA	
Acanaloniidae (acanaloniid planthoppers)	Mexico
Aleyrodidae (whiteflies)	
Aleyrodes spiraeoides Quaintance	CA
Bemisia tabaci (Gennadius)	CA
Trialeurodes vaporariorum (Westwood)	CA
[as *Trialeurodes vaporariosum*]	
Aphididae (aphids)	
Aphis gossypii Glover	CA
A. helichrysi Kaltenbach	CA
[as *Anuraphis helichrysi* Kalt.]	
Macrosiphum spp.	CA
[as *Macrosyphum* spp.]	
Myzus persicae (Sulzer)	CA, TX
Pentalonia nigronervosa Coquerel	CA
[as *Pentalonia nigronerosa*]	
Rhopalosiphum nymphaeae (Linnaeus)	CA
[as *Rhopalosiphum nymphaeal*]	

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
HOMOPTERA (continued)	
Cicadellidae (leafhoppers)	
Carneocephala fulgida Nottingham	CA
Draeculacephala minerva Ball	CA
Empoasca arida DeLong	CA
Empoasca spp.	Mexico, TX
Macrosteles divisus (Uhler)	CA
Scaphytopius heldoranus (Ball)	Mexico
[as *Cloanthanus heldoranus* (Ball)]	
Diaspididae (armored scales)	
Lecaniodiaspis sp.	Mexico
Rhizaspidiotus dearnessi (Cockerell)	AZ, Mexico, NM, TX
[as *Targionia dearnessi* Cockerell]	
Targionia yuccarum (Cockerell)	Mexico
Targionia sp.	Mexico
Flatidae (flatid planthoppers)	Mexico
Kerridae (lac scales)	
Tachardiella cornuta Cockerell	Mexico
Ortheziidae (ensign scales)	
Orthezia sp.	Mexico
Pseudococcidae (mealybugs)	
Phenacoccus gossypii Townsend & Cockerell	CA
Pseudococcus spp.	CA
[as *Pscudococcus* spp.]	

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
HOMOPTERA (continued)	
Pseudococcidae (continued)	
Puto yuccae (Coquillett)	Mexico
[as *Ceroputo yuccae*]	
HYMENOPTERA	AZ, CA, Mexico, NM, TX
Cynipidae (gall wasps)	TX
[as gall-flies]	
Formicidae (ants)	TX
Atta texana (Buckley)	TX
Pogonomyrmex barbatus (F. Smith)	NM, TX
Pogonomyrmex spp.	CA
Solenopsis xyloni McCook	CA
[as *Solenopsis xyloni* var. *maniosa* Wheeler]	
Mymaridae (fairyflies)	
Anaphes ovijentatus (Crosby & Leonard)	AZ
Pteromalidae (pteromalids)	
Halticoptera aenea (Walker)	CA
Syntomopus americanus Ashmead	CA
ISOPTERA (termites)	
Termitidae	
Ganthamitermes tubiformans (Buckley)	TX
[as *Amitermes tubiformans* Buckley]	

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
LEPIDOPTERA	
Arctiidae (tiger moths)	
Estigmene acrea (Drury)	CA
[as *Estigmene acraea* (Drury)]	
Hyphantria cunea (Drury)	CA
[as *Hyphentria cunes*]	
Lymantriidae (tussock moths)	
Dasychira mendosa (Hübner)	
Lyonetiidae (lyonetiid moths)	CA, Mexico
Bucculatrix spp.	Mexico
Noctuidae (armyworms, cutworms, etc.)	Australia, CA, TX
Heliothis spp.	Australia
Mamestra picta (Harris)	TX
[as *Mamestra picta* Linne]	
Spodoptera sp.	CA
[as *Laphygma* sp.]	
Trichoplusia ni (Hübner)	CA
Nymphalidae (brushfooted butterflies)	Mexico
Pyralidae (pyralid moths)	
Achyra rantalis (Guénee)	NM, TX
[as *Loxostege similalis* Guénee]	

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
LEPIDOPTERA (continued)	
Tortricidae (tortricid moths)	
[as **Olethreutidae**]	
Eucosma sp.	Mexico
Platynota stultana Walsingham	CA
NEUROPTERA (lacewings)	Mexico
ORTHOPTERA	
Acrididae (short-horned grasshoppers)	AZ, CA, Mexico
Encoptolophus pallidus Brüner	Mexico
Melanoplus cinereus Scudder	CA
[as *Melanoplus cinereus cyanipes* Scudder]	
M. differentialis (Thomas)	CA
M. femurrubrum (DeGeer)	CA
[as *Melanoplus femur-rubrum* DeGeer]	-
M. marginatus (Scudder)	CA
M. devastator Scudder	CA
[as *Melanoplus mexicanus devastator* Scudder]	
M. sanguinipes (Fabricius)	AZ
[as *M. mexicanus mexicanus* (Saussure)]	
Oedaleonotus enigma (Scudder)	CA
Platylactista aztecus (Saussure)	Mexico
[as *Platylactisca azetca* Guerney (Saussure)]	
Trachyrhachis kiowa (Thomas)	Mexico
[as *Trachyrhachys kiowa* (Theo.)]	

Appendix 1. (continued).

Taxonomic grouping	Some areas of occurrence
ORTHOPTERA (continued)	
Gryllidae (crickets)	
Gryllus sp.	CA
[as *Acheta* sp.]	
THYSANOPTERA (thrips)	
Thripidae (common thrips)	
Aeolothrips sp.	CA
[as *Aelothrips* sp.]	
Chirothrips aculeatus Bagnall	CA
Frankliniella minuta (Moulton)	CA
F. occidentalis (Pergande)	CA
[as *F. occidentalis trehernei* Merg.]	
[as *Frankliniella moultoni* Hood]	
Microcephalothrips abdominalis (D. L. Crawford)	India
[as *Microcephalo-thrips abdominalis*]	
Thrips tabaci Lindeman	CA

Chapter 9

Influence of Environment and Management Practices on Rubber Quantity and Quality

Francis S. Nakayama

ENVIRONMENTAL EFFECTS ON PLANTS

Natural Habitat

An understanding of the natural habitat of the guayule plant (*Parthenium argentatum* Gray) may provide background information on how this habitat can be used in the cultivation of the shrub for commercial natural rubber production. Guayule grows naturally as isolated patches in the semiarid Chihuahuan Desert region of north central Mexico and next to the Big Bend area of Texas in the southwestern United States. It is located on calcareous foothills and outwash slopes where it can compete best against weeds and grasses. The native habitat is confined to an area bounded by about 23 to 31 degrees latitude and -100 to -105 degrees longitude, most of which is located in Mexico.

The guayule habitat ranges in elevation from 750 to 2,000 m, in temperature from 3 to 34°C, and in rainfall from 130 to 350 mm. Precipitation occurs mainly in the late spring and early fall; distribution varies. Extensive details covering climatic data and analysis of the region are presented in *Guayule in Australia* by Stewart and Lucas (1). Their objective was to translate information about the native habitat for selecting sites to optimize guayule rubber production in Australia. In the United States, where the most extensive studies on guayule commercializa-

tion are being carried out, guayule cultivation trials are being conducted slightly outside the native boundaries to the 32 to 36 degrees latitude and 94 to 124 degrees longitude zone (Figure 1). These regions have different rainfall distribution patterns and receive different quantities of rain (usually less); temperatures are higher.

Bullard (2) delineated the areas of the four southwestern states in which guayule may be grown based on temperature and precipitation (Figure 1). In developing the delineations, more concern was given to minimum rather than maximum temperatures. A temperature minimum

Figure 1. Potential areas for guayule cultivation in the United States, after Bullard (12).

less than -15°C was considered unsuitable. Mitchell (3) found that the guayule plant could be damaged when exposed to temperatures of -10°C for only a few hours unless the shrub had been acclimatized to low temperature before exposure. He also noted varietal differences in the threshold minimum low temperatures. Minimum-maximum precipitation range was set at 280 to 900 mm, respectively. Irrigation can be used to make up for the lower rainfall to increase yield and to bring the shrub to earlier maturity than the native stands. Bullard (2) also indicated that rainfall distribution was taken into account with preference placed on maximum rainfall occurring in the early spring and summer and minimum in the late fall and winter. Uniform rainfall distribution combined with a warm winter was considered unsuitable because the combination did not provide a dormant period, which is necessary for rubber accumulation.

McGinnies (4) describes the perennial guayule plant as growing best between 30 and 38°C, with restricted growth below 15°C. Flowers and fruits are produced as long as conditions are favorable, and, thus, the plant does not follow a definite seasonal pattern. Flowering of the native plant occurs any time depending on the amount of rainfall and daylength. Irrigated plants continue to flower from early spring to late fall.

In a detailed survey of a natural habitat around Saltillo, Mexico, Ostler and Adler (5) noted two distinguishing aspects of the soil environment. First, the soil phosphorus content was high and, second, the soil depth was shallow, about 12 cm in the limestone parent material with a confining caliche layer. They concluded that rubber content was influenced primarily by soil characteristics, particularly well-drained soil.

Past Research

When we look back at the history of guayule research, we see limited activity probably because its commercialization as a major crop was not an important factor. The Emergency Rubber Project (ERP), during the World War II era of 1941 to 1945, drastically changed the research atmosphere. The effort then was directed toward the immediate increase in rubber, but again

with no goal for eventual commercial production. The war effort provided almost unlimited scientific manpower with adequate technical and physical support. The major part of the guayule agronomic and processing research during this period was conducted around Salinas, California (Figure 1), with minor experimental plantings in southern California, southern Arizona, southwestern and southeastern New Mexico, and western Texas. Salinas has a milder climate and higher rainfall than guayule's natural habitat. Guayule grew well and produced adequate seed in this region, and much of the breeding and agronomic management experiments began during this period. Unfortunately, all the cropland and plant material and much of the experimental data were lost immediately following the end of the war. Other data appear in unpublished reports, which are difficult to obtain. Except for some research efforts in mid-1950, new studies on guayule did not start until the late 1970s, and agronomic research reports are just starting to appear in the scientific literature. Thus, to be as current as possible, recent abstracts and unpublished materials will be included in this chapter.

Survival of guayule under the semidesert condition is attributed to its tolerance to extreme water stresses of a long duration. Ehrler and Nakayama (6) noted that established plants undergo partial dormancy and can recover very rapidly when water becomes available. Transplants and seedlings in particular are susceptible to moisture stresses like other field crops. Irrigation provides the means for stress management that could be used to improve rubber quantity and quality. Benedict, McRary, and Slattery (7) suggested that alternate low and high moisture stress should be imposed on the guayule plant based on the approach that rubber synthesizing and storage tissue production will be promoted during the nonstress period and rubber synthesis during the stress period.

There is an important aspect to water availability for the guayule plant. Guayule grows more slowly than other plants so that weeds can readily outgrow and overwhelm seedlings when water is available. This is of particular importance in direct seeding where young seedlings cannot compete against native weeds. In contrast, native guayule with limited moisture can survive, whereas, competitive weed growth and survival is restricted.

Yield Functions

The final processed rubber yield from guayule will depend upon the interactions of many variables. The more important ones are indicated in the following relation:

YIELD = f/[CLIMATE (temperature, rainfall, radiation),
CROP (variety),
SOIL (physical, chemical, biological),
AGRONOMIC MANAGEMENT (plant establishment, population, water, fertility, insect, disease, weed, harvest date, plant age, post-harvest treatment),
PROCESS MANAGEMENT (rubber, resin, coproduct)].

Some of the variables listed in the functional relation are of sufficient importance that they are covered separately in other chapters in this publication. While the rubber extraction process appears to be separate from that of agronomic management, the physical condition of the shrub, the ratio of leaf to stem, and the moisture, resin, and rubber contents all have an impact on the extraction efficiency.

Benedict (8) indicated that the factors directly or indirectly affecting rubber yield were temperature, nutrient availability, light intensity, and season, all of which relate to the plant's response to its environment. Guayule plants grown with the highest temperature, light, and nitrogen level produced the highest total rubber. They had the highest dry-matter yield (excluding leaves), but not necessarily the highest percentage of rubber. Mitchell, Whiting, and Benedict (9) observed that shading, even at 25 percent light-intensity reduction, significantly decreased stem and root growth. Rubber concentration was decreased by 25 percent and total rubber per plant by 35 percent. In contrast, shading improved plant growth in infertile soil. In

the winter, plants grown under high light intensity had more functioning leaves than those grown under low intensity. Reduced light intensity favored seed production during the summer months. MaCrae, Gilliland, and Van Staden (10) in laboratory studies showed that rubber synthesis was highest in plants exposed to the highest light intensity.

Phytotron data of Downes and Tonnet (11) support the field observations that high temperature and high radiation promoted shoot growth, whereas high temperature and low radiation restricted growth. They also observed that plants grown continuously in the day-night temperature environment of 18 to 10°C had higher rubber contents than those grown at 25 to 19°C. Bonner (12) had earlier shown that night temperatures on the order of 7°C were the most effective in increasing rubber content. MaCrae, Gilliland, and Van Staden's (10) data also indicated that incorporation of rubber precursors was best with low night temperatures.

The effect of soil temperature on guayule growth was investigated by Benedict (8). He maintained a constant air temperature of 21 to 32°C, night and day, with varying soil temperatures of 4 to 32°C and found the optimum growth occurring between 27 to 29°C soil temperature. Rubber percentage was highest in plants grown at the lower soil temperatures, but the yield of rubber was greatest for the plants grown at the highest temperatures because of their larger biomass.

The primary factors such as climate and soil are considered as invariable from the management standpoint, except that site selection may be critical since its environmental limits must be within the growing regime of the guayule plant. Management can adjust for the lack of rainfall, poor soil physical structure relating to drainage or aeration, soilborne pathogens, and low nutrient level; it can select varieties that are drought tolerant, adapted to low temperature or radiation, and resistant to disease.

Nutrition

Most of the nutrient studies on guayule were conducted during the Emergency Rubber Project-era of the 1940s. Guayule does not respond as spectacularly to the applications of major elements (nitrogen, phosphorus, potassium) as do other existing agronomic crops. Also, nutrient deficiency symptoms are difficult to recognize and, thus, have not been established for guayule. Tingey (13) obtained little increase in yield of rubber with fertilization. McGinnies (14) went as far as stating that fertilizers may depress rubber formation. However, in nutrient culture studies, it was shown that growth and rubber accumulation decreased with decreasing available nitrogen and phosphorus (15, 16). Nutrient treatment combinations that tended to grow the largest plants tended to produce plants with the lowest rubber concentration. In contrast, the smallest plants did not necessarily have the highest rubber concentrations. Gravel culture studies of Thomas (17) showed an increase in plant size and rubber content with an increase in phosphorus application. There was a leveling of yield when sufficient phosphorus was available to the plant. Apparently, most guayule field plantings in the past were made on soils of sufficient fertility so that nutrient deficiency was not a serious problem.

High ratios of ammonium to nitrate nitrogen decrease both growth and rubber percentage (15). Hammond and Polhamus (18) cite an unpublished 1945 report of Davis who found that plant growth response was better with the application of nitrate nitrogen than ammonium nitrogen or manure in sandy Yuma soils with low fertility. In similar soil and climatic conditions 40 years later, Bucks et al. (19) obtained maximum rubber yield with high nitrogen applications, but had to accomplish this in conjunction with high water applications.

Bonner (15) further observed that the guayule plant did not respond to sulfate deficiency. When calcium, potassium, and magnesium concentrations were low, growth and rubber accumulation were depressed, but this was negated if magnesium concentration was high.

Boron deficiency was the most readily demonstrated of all the minor elements (20). Plants with such deficiency showed reduced growth and rubber concentration and could recover adequately when supplied with boron.

While salinity is not perceived as a nutrient problem, it can affect plant growth in a negative sense due to the overabundance of various cations and anions that change the osmotic and nutrient balance of the plant. Salinity greatly increases the mortality of young guayule seedlings. Miyamoto et al. (21-23) attributed this problem of salinity to the high salt accumulation at the soil surface that can drastically injure the hypocotyl of the emerging seedling. Transplants, on the other hand, are much more tolerant of salinity than seedlings (24). Mihail, Alcorn, and Ray (25) found a positive interaction between the salinity of irrigation water and plant mortality due to the fungus disease of guayule *Macrophomena phaseolina*. Wadleigh and Gauch (26) showed that high sodium concentration affected guayule growth much more than calcium. This was also observed in laboratory germination studies by Ballal and Daugherty (27) where sodium had a detrimental effect compared to calcium and also chloride over nitrate.

Conversely, the data of Maas et al. (28) show that the guayule plant, after establishment, is highly salt tolerant. Their yields were not harmed at soil electrical conductivity of 16 dS/m, whereas other commercial crops that are considered salt tolerant have salinity threshold values of 9 dS/m and less. Unfavorable salinity effects were due primarily to plant mortality. Their results also indicate that low levels of salinity may be beneficial for guayule growth and rubber production. Retzer and Mogen (29) reported earlier that excess salt of 0.3 percent retarded growth and that concentrations above 0.6 percent killed the plant. The rubber content of salt-stressed plants tended to be higher than that of nonstressed ones.

Seasonal Rubber Production

Guayule, under cultivation, exhibits a distinct seasonal cyclical pattern of alternate growth and rubber accumulation. Biomass production is greatest in the spring and fall months, and rubber

accumulation is highest in the winter months. Hunter and Kelley (30) observed that there was no increase in dry weight of the guayule shrub between November and March for plants grown in Shafter, California. Bucks et al. (19) reported that the seasonal gains followed a stair-step relationship with time, with the flat portion of the growth pattern occurring in the summer and winter periods. The rubber yield behavior as related to time of sampling is illustrated in Figure 2, where the yield line represents the average of six irrigation treatments and three cultivars. The vertical line at each of the harvest dates designates the range between the highest

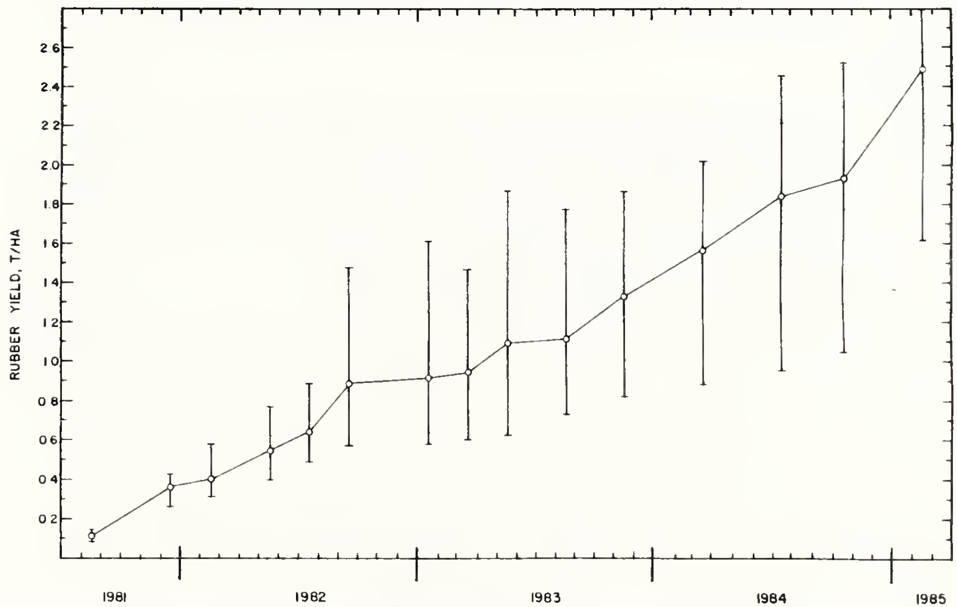

Figure 2. Seasonal guayule rubber production under irrigation at Mesa, Arizona.

and lowest irrigation treatments. The optimum yearly harvest, which covered a four-year cultivation period, was estimated to be in the January to March interval.

Hager et al. (31) concluded that young plants can synthesize rubber molecules with molecular weights as high as the mature plants, but that younger plants also have molecules of low molecular weight. From their molecular weight distribution and electron micrograph data, they suggested that there are two stages in rubber growth through the polymerization process where: a) the size of rubber droplet does not allow further polymerization and b) the repeated polymerizations up to that molecular weight tend to make the most probable molecular weight distribution. This would imply that there may be a limit to the maximum molecular weight in the guayule plant.

Other plants like the milkweed (*Asclepias syriaca*) also synthesize low-molecular-weight rubber particles, but their increase to the high molecular weight through polymerization does not occur because the plant dies. Guayule, on the other hand, can continue to form new starter rubber nuclei and continue to polymerize them to form larger particles throughout its perennial growth cycle. The induction and increase in rubber formation have been related to a stress period, which can be induced under natural or managed conditions.

Meeks, Banigan, and Planck (32) indicated a seasonal influence on the molecular weight of rubber yield. During the January to May period the molecular weight increases with the peak in mid-May, followed by decreases from June through August. The presence of low-molecular-weight polymer is pronounced during the period of active growth in the spring and early summer and is absent in the quiescent winter months (33). Garrot, Schloman, and Ray (34) observed that the highest rubber quality was present at the end of the cold-stress period, extending to late May. In another set of experiments of shorter duration (January to August), Schloman, Garrot, and Ray (35) measured lower rubber and resin contents during June and July. Plants grown at greater water stress had higher-molecular-weight rubber in January and June than the nonstressed plants.

Water stress definitely affects rubber yield as shown in Figure 3, where yield and crop water stress are essentially linearly and negatively related. Crop water stress is based on relating the canopy temperature of a crop at any given time to the canopy temperature of the nonstressed, adequately irrigated crop (36). Unpublished data of Cornish and Backhaus (37) indicate that rubber transferase activity, which is essential for rubber synthesis, is higher in drought-stressed plants than nonstressed plants, suggesting the possibility of higher rubber synthesis when the plant is under stress. However, nonstress periods are important for the formation and accumulation of rubber storage cells and photosynthates needed for eventual rubber production. Crop

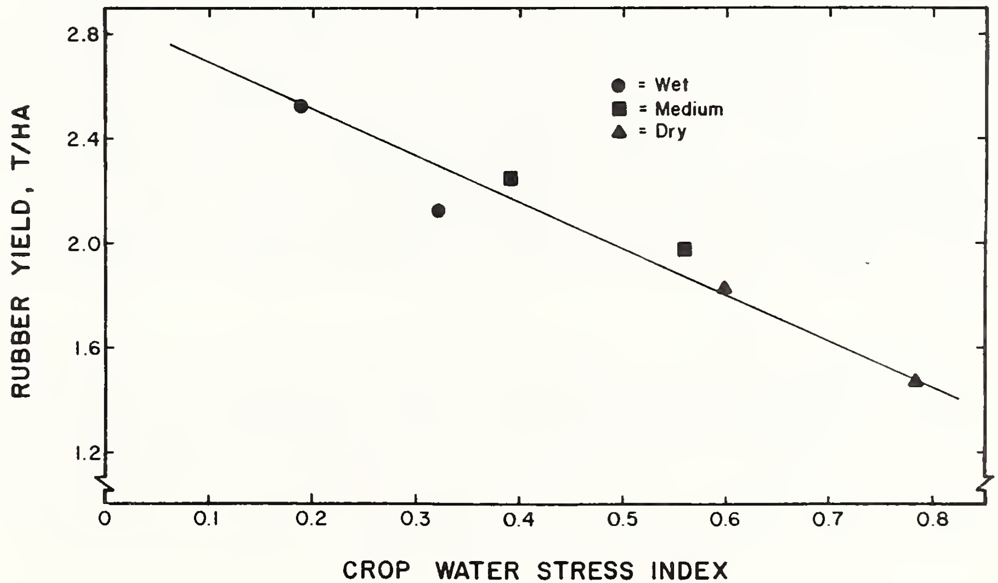

Figure 3. Water stress effects on rubber yield of four-year-old shrub.

water stress indexing would be a useful tool for scheduling irrigation to control stress and also for making predictions of rubber yields. An optimum balance between stress and nonstress periods is needed, the former to promote rubber formation and the latter to increase biomass.

Plant Age and Population

With the exception of clipped plants, the earliest harvest period for guayule shrub, with existing varieties, is after two years of plant establishment. This time period is possible where water supply is adequate for ample plant biomass production. Thus, under irrigated culture, the minimum harvest time would be two years, whereas, under dryland conditions, the time period would be on the order of four to five years or longer. Some of the interrelations are shown in Figure 4, which compares yield to harvest date, location, and population. Rubber yields at Yuma under adequate moisture conditions (Figure 4A, small open circle) are significantly higher than under continually stressed conditions (solid circle). To obtain similar two-year yields, the stressed plants must be grown for an additional two years (38). Yields were also higher in Yuma than in Mesa (cross) because of a longer growing period and light intensity at Yuma than at Mesa. Plant populations were 49,500 and 54,000 per hectare at Yuma and Mesa, respectively.

A series of plant population studies were conducted in Salinas, California, in an attempt to increase yield and decrease the growing period. The data of Tingey and co-workers (13, 39) are presented in Figure 4B, comparing the rubber yields of three populations of 11,200 (LP), 37,300 (MP), and 1,088,000 (HP) plants per hectare. The largest population had the highest yield. The results presented were for adequately irrigated plots. Thus, by increasing plant population we can expect acceptable yields in a shorter growing interval. However, we need more data on high-density planting to see whether such an approach would help in solving the problem of obtaining high yields. We also must consider the fact that availability of seed is limited and that harvesting difficulties may occur. We do not have information on the self-

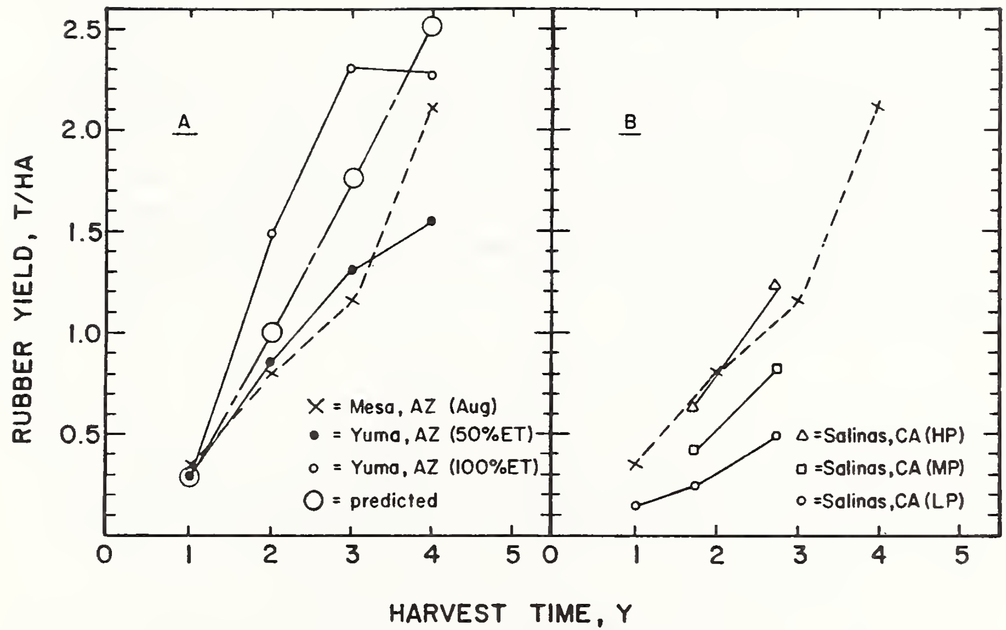

Figure 4. Interrelation of rubber yield with location, irrigation, harvest date, and population. Large circle in A represents computed values from a regression equation; small open and solid circles represent data from Yuma, Arizona, at 100 percent evapotranspiration (ET) and 50 percent ET, respectively; crosses represent rubber yield at Mesa, Arizona. Part B covers plant population effects at 11,200 (LP), 37,000 (MP), and 1,000,000 (HP) plants per hectare.

thinning process that would control the upper limit of plant population. Site selection may be another solution—the yield data from Mesa, Arizona, with a plant population of 54,000 per hectare, show similar values as data from Salinas, California, with nearly one million plants (Figure 4B). Unquestionably, yields can be significantly increased both at the Yuma and Mesa sites with higher plant densities and where water availability is not limited.

Stewart (40) proposed several equations that may be used to estimate rubber yields. These relations take into account plant densities, time of harvest, and environmental factors. The latter component is empirically selected, and equations must be developed for the particular environmental or management conditions. In Figure 4A, the equation developed for the "favorable" condition (40) is described by

$$Y = [1.359(t-0.629)]/[1 + 43285/d],$$

where Y (rubber yield), t (time), and d (plant density) were used to calculate regression line. This equation defines a linear relationship between rubber yield and time of harvest. These relations would be useful when fully implemented for the specific locations, varieties, and management practices to predict yields for use in economic projections and management.

A computer model for guayule has been proposed by Kimball (41) based on relations between weather, soil, and various plant characteristics and processes that can be described mathematically (photosynthesis, transpiration, age, variety). The simulation model can be used to predict plant temperatures and stress, water losses, and soil water distribution on an hourly basis and biomass and rubber production on a daily basis. With assumptions on climatic parameters and irrigation applications, projections concerning yield can be made on a seasonal basis. Unfortunately, the model cannot yet be used as required plant and yield parameters are unavailable at present.

Harvesting of Guayule

The results for rubber accumulation in guayule suggest that the optimum time for whole-plant harvest is during the winter to early spring months, when the rubber content is the highest. Under irrigation, the plant age would be at least two years, and, with no irrigation and limited rainfall, the plant age would be four years or older. An alternative to whole-plant harvesting is clipping or pollarding, which was first suggested by Lloyd (42). The ratoon crop can regenerate to produce new growth in succeeding years without the need for replanting to improve the economics of guayule culture. Clipping height and date are very critical. Foster et al. (43) obtained 100 percent regrowth after clipping on four-year-old guayule plants when they were cut 10 cm above ground and suggested that clipping should be done during the dormant or cool season. Unpublished studies also indicate that regrowth was poorest when plants were clipped in the summer months. Estilai and Waines (44) were able to clip at ground level and found that shrub survival and regrowth rates varied with the different varieties. Both high salinity of the irrigation water and soil increased the mortality of shrubs after they were clipped (21).

Experimental data comparing clipping to whole-plant harvest yields are limited because such studies involve long cultivation periods. Also, comparison of yields from a set of years are difficult to make because of variations in climate from year to year. Ray et al. (45) cite the ERP work of Hunter, Burtch, and McDowell who obtained higher rubber yields for clipped plants based on cumulative totals than for whole unclipped plants of equal combined ages. The comparison was between whole plants that were grown for two years, harvested, replanted, and grown for another two years versus plants that were grown for two years, then were clipped, allowed to grow for two years, and then harvested as whole plants. Similar types of studies were conducted by Ray et al. (45) using the two-year harvesting scheme. Yearly clipping, however, had an adverse effect on the plants. Our studies—which were based on the Yuma, Arizona, studies (unpublished) as part of the main experiment reported earlier—show that the single fourth-year whole-plant harvest had slightly more total biomass, rubber, and resin yield

than either the cumulative harvest of second-year clipped plants plus the additional two-year regrowth (second harvest involved the whole plant) or the third-year clipped plants plus the additional one-year regrowth.

Rubber Degradation

Curtis (46) observed quite early that guayule rubber was converted to acetone-soluble materials when the branch of the plant was exposed to field conditions. According to Keller and Stephens (47), guayule rubber is degraded by the resin constituent, most probably the unsaturated fatty acid, linoleic acid. The oxidation of unsaturated fatty acids forms hydroperoxide which in turn initiates the degradation of the rubber molecule. Linoleic acid can also act as a chain-transfer agent and radical scavenger in the thermal degradation process. Bhowmick, Rampalli, and McIntyre (48) noted that the degree of degradation based on molecular weight was higher the greater the number of double bonds in the unsaturated fatty acids, that is linolenic > linoleic > oleic acid. Stearic acid, a saturated fatty acid, had the lowest rate of degradation. The rate of degradation for the fatty acids was first order relative to the concentration of the respective acid. Keller, Winkler, and Stephens (49) reported that a nonsaponifiable fraction present in the resin appeared to retard rubber degradation. Rubber degradation can be retarded by cold storage. Black, Swanson, and Hamerstrand (50) found little change in rubber molecular weight when the shrubs were stored in the freezer for one year.

Antioxidants (AO) are added to the guayule-solvent mixture to prevent rubber degradation during the extraction process. However, adding AO to the raw material just after harvest prior to storage or during transport to the processing facility does not appear to be economically feasible. No effective means are available to bring the AO in contact with the rubber molecule in the plant cell.

Post-harvest Handling of Guayule

Adequate information is lacking as to how the harvested shrub should be handled prior to processing. We must presume that some degree of degradation would occur based on laboratory studies of rubber degradation and also on analogy to other field crops, but the extent of the change and how it would affect processing are not yet known. Hammond and Polhamus (18) reported that during the Emergency Rubber Project, the shrub was dug, windrowed, cured for three to five days to remove surface moisture, baled into 90.7-kg (200-lb) bales, and shipped by trucks or rail to the mills. They indicated no deterioration of rubber during transportation over long distances. The plant material was stored under cover for 10 to 12 days before processing. The limited study of Taylor and Chubb (51) showed that fresh shrub gave the best rubber yield and highest molecular weight, followed by shrub that was defoliated and stored. An increase in recovered rubber occurred following storage between three to six weeks and then decreased with longer storage periods. The nondefoliated shrub with storage gave the lowest rubber yield and quality, but showed a slight increase in resin yield.

Unpublished data of Dierig et al. (52) show a decrease in rubber content and molecular weight after field storage of individual plants for six weeks in central Arizona during the spring months. The degradation was minimal for the first two weeks, but increased after that. Data indicated that there was a varietal difference in the rate of degradation.

Wagner et al. (53), while developing a process for extracting guayule rubber, obtained rubber that was soft and tacky from the Brawley, California, shrub. They thought that this condition possibly could be attributed to the degradation of the latex when the shrub was stored for two weeks at summer ambient temperatures. In contrast, shrubs from other locations when stored under refrigeration for up to eight months gave rubber that was rigid and nonsticky.

The present scenario is to harvest the shrub by clipping or rooting out the whole plant. From there, the harvested material is stored in the field as whole plants and either chopped in the field, left as whole plants, or baled and then transported to the extraction facility for processing.

Some field drying may be required to meet the needs of the processing facility. This specification would depend upon the extraction process being used. The restriction on optimal water content would not be as critical for the flotation process as for the direct chemical extraction process, except possibly where grinding requires a specific moisture content. For the flotation process, the method developed by Gutierrez and Kay (54) can be used involving the temporary storage of ground shrub in water to control deterioration; the adjustment of solution pH to less than 5.0 or greater than 8.5 can be used to control odor.

Since the stems contain the bulk of the rubber, defoliation would be desirable. Some leaves break off after the shrub is partially dried, but if drying cannot be accomplished because of location, then chemical defoliation may be desirable before plant harvest. The guayule plant does not form an abscission layer as other deciduous plants so that the leaves remain attached to the plant. Van Staden and Gilliland (55) were able to produce leaf fall with applications of high ethephon concentration (5 g/l) to the plant. More research is needed in the area of defoliation to improve processibility.

SUMMARY

Guayule, like other major crops, can be successfully grown outside its native habitat. Optimum conditions for maximum yields include high light intensity, high day with low night temperatures, and adequate moisture and fertility. Development of higher-yielding varieties will help considerably in achieving guayule commercialization. For maximizing production, irrigation appears to be the best cultural management tool available offering the possibility of planting at maximum density. Drought tolerance of the plant permits better flexibility in stress management for increasing rubber content in the plant tissue. Adequate fertility is needed, but is not as critical for guayule as for other field crops. Optimum time for harvest is during the late winter to early spring months when rubber synthesis has achieved the maximum storage of rubber in

the plant for that growing cycle. Since rubber degradation begins soon after harvest, plants must be processed as soon as possible or adequate control steps taken to reduce the rate of degradation. Thus, it is apparent that proper coordination between field and rubber extraction operations is important.

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Chapter 10

Guayule Harvesting Equipment

Wayne Coates

SEED HARVESTING

Crop Characteristics

Guayule is a perennial, normally setting the majority of its seed twice annually. Seed can first be harvested when plants are only 0.3 m tall; older plants reach 1 m in height. Seeds grow in clusters, referred to as inflorescences, on peduncles or small stalks that extend outward in all directions from the plant. The inflorescence forms a spherical surface from which the seed must be removed. When planted in rows, the proximity of other plants results in seeds being produced only on the top and sides of the row.

Since the seed does not set uniformly, several collections, spaced at appropriate intervals, are required to maximize yields. This situation demands an exceptionally gentle, nondestructive harvesting method to avoid damage to seed heads that are in the flowering or filling stage. Guayule seed shatters readily in low humidity, making its removal very easy, but also significantly increasing the possibility of weather and harvesting losses. Under high humidity, the seed is very difficult to dislodge so harvesting must be limited to late morning and early afternoon. Complicating these harvesting problems are the relatively low yield of 1.1 kg/ha, the very small size of the seed (1,000 seeds/g), and the low percentage of clean seed to bulk weight of material harvested (0.5 to 2.7 percent).

Seed Harvesters

Several seed harvesters were developed from 1945 to 1955 (1). One relied solely on suction to remove the seed from the plants, and others used either revolving beaters or brushes to dislodge the seeds into troughs that passed below the branches. All of these systems had very low harvesting efficiencies. In 1950 a harvester was developed that dislodged seed from the plants with a beater and then sucked it from the ground through nozzles shaped to conform to the irrigation furrows (1). Although harvesting efficiencies were very high, large amounts of soil and trash were picked up with the seed. Tysdal (2) reported on the development of a harvester that consisted of a hood to cover the plants, a vibrator to dislodge the seed, and a blower to initiate seed movement toward suction inlets. No indication of harvesting efficiency or speed of operation was reported for this device.

Coates (3) described seed harvester development efforts at the University of Arizona. An early prototype employed a rotating brush to dislodge the seeds, but this was found to be much too aggressive, extensively damaging the plants and collecting excessive trash. Later development centered on using suction combined with impact to the inflorescence to remove seed from the plants.

Extensive field testing of various prototypes led to the development of a harvester design that has been used in Arizona, California, New Mexico, and Texas. The basic design requirements established during the development program were a) good operator visibility; b) ground clearance in excess of 75 mm; c) adjustable heads to accommodate plants from 30 to 90 mm in height; d) providing suction without having the seed pass through fans; and e) harvesting efficiency greater than 90 percent. Several of these design objectives were met by using a cotton picker for the basic power unit. The high operator's platform provided good visibility, and the frame gave adequate shrub clearance. The suction was provided by modifying the pneumatic conveying system used on the cotton picker. Pressurized air at a high velocity was forced through a nozzle placed along the inside surface of a section of pipe, thereby creating a

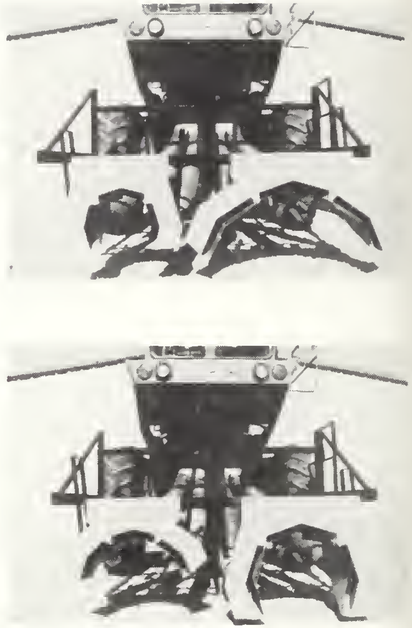

Figure 1. Large and small seed-harvesting heads. Top photo shows the heads fully open, the bottom view shows the heads fully closed.

Figure 2. Harvesting seed in a two-year-old field of guayule.

venturi effect. Suction was produced in the pipe on the upstream side of the nozzle, while downstream, the pressurized air and material picked up during harvesting was discharged. Air flows from 3,570 to 4,250 m^3/hr at 645 Pa vacuum were available with this system.

The harvesting heads, shown in Figure 1, were composed of three sections. The side sections were attached by hinges to the center or top section. Two venturies supplied suction to the head. One was connected to the top section, and the second supplied equal amounts of suction to the sides. This design was developed to take advantage of the fact that the majority of the seed is located on the top of the plants, rather than on the sides. Changes in the gross shape and size of the heads could be achieved in either of two ways: the side sections could be pivoted in or out or the smaller 250-mm-wide sections, either the top or sides or both, could be interchanged with the larger 750-mm-wide sections. Various attachments were also developed for the leading edge of the sections. These helped to improve harvesting efficiencies under different field conditions.

The two-row harvester, shown in Figure 2, had a seed hopper with a screen top, which allowed dust and air to escape. A sloped bottom permitted unloading directly into sacks or other suitable containers. To reduce operator fatigue and to eliminate machine or plant damage resulting from running the heads too close to the ground, an automatic head height control was incorporated into the design. The system could be manually overridden to skip over trash or weeds, or when larger plants were encountered.

Field trials yielded harvesting efficiencies ranging from 90 to 98 percent for ripe seed under favorable harvesting conditions. The ratio of clean seed to bulk weight of material harvested varied from 0.5 to 2.7 percent. Ground speeds during harvest ranged from 2.0 to 3.2 km/h, with the optimum found to be from 2.4 to 2.7 km/h. Average theoretical field capacity for the harvester was 0.5 ha/h.

Although general performance of the harvester was adequate, performance was reduced by adverse harvesting conditions such as humidity extremes. High humidity makes the seed difficult to dislodge from the plants, thereby reducing the number of harvesting hours available

during the day and increasing the chances of weather losses. Low humidity causes natural seed shattering and also allows some seed to be knocked from the plants in front of the head and lost during harvesting. To improve harvesting efficiencies during adverse conditions, additional development work was undertaken at the University of Arizona.

Coates (4) reported on the design of a harvesting head that combines multiple impacts with scraping to dislodge the more firmly attached seed; in addition a moveable collection surface passes under the plants and catches seed that is dislodged but fails to be collected by the suction. Figure 3 is a schematic of the device, and Figure 4 shows the prototype, which was fabricated and field tested. At the bottom of the head, two rows of moveable plates (A) are set at an angle with respect to the soil surface in order to funnel seed from the center of the head to the side. Each plate is held in a neutral position by a spring attached to the plate bottom. When the leading edge of a plate contacts a stalk or branch, it moves rearward and outward, and then, when the obstruction has cleared the section, the spring returns the plate to the neutral position. A pan (C) on either side of the head collects the seeds as they fall from the plates. A duct (D) located above the pans provides the suction to lift the seeds into the pneumatic conveying system, which then carries them to the seed tank.

The impact and scraping action used to dislodge seed from the plant is provided by two components. A rectangular-shaped suction opening provides the impact and scraping on the plant tops. This section is mounted on a hydraulic cylinder, which is controlled from the operator's platform. By raising or lowering the height of the section relative to the plant tops, the operator can decrease or increase the aggressiveness of the head while the moveable plates remain at a set height relative to the surface of the bed. Five vertical rods spaced along each side of the head provide the second means of dislodging seed. As the center section moves up and down, the rods slide through guides mounted on it and subsequently move inward or outward depending upon the location of their lower ends. This provides a more or less aggressive harvesting action as required.

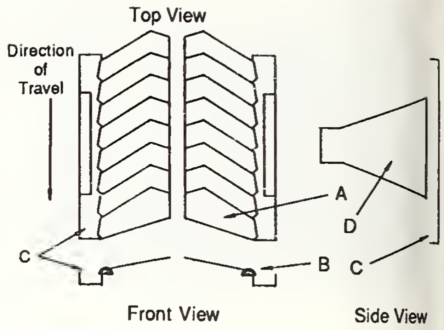

Figure 3. Schematic of seed-harvesting head. A - pivoted plates, B - plate angle adjustment point, C - side collection pans, D - seed-conveying duct.

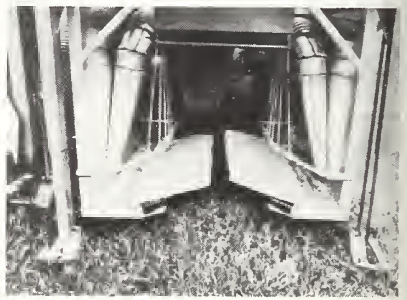

Figure 4. Prototype seed-harvesting head.

To evaluate harvester performance and compare harvesting head efficiencies, field trials were conducted in three-year-old guayule. To minimize field variability, the two types of harvesting heads were operated simultaneously on adjacent rows. Table 1 presents the mean results from two replicates on each of four varieties of guayule.

Table 1. Guayule seed harvester performance.

Variety	Ground speed (km/h)	Yield (kg/ha)	
		Suction head	Plate head
12229	1.8	2.79	2.87
N576	1.8	4.31	5.38
11605	2.5	1.69	3.09
N565	2.5	7.78	7.77

The harvesting data show that in all but one case the plate head improved harvesting efficiency. Germination trials indicated that seed quality was equal for both heads, thus dispelling concerns that the more aggressive action of the plate head removed immature seeds from plants.

Seed Harvesting from the Ground

The nonuniform maturity of guayule seed, combined with finite machine capacity, prevents large fields from being harvested before some shattering takes place. In addition, strong winds and severe rainstorms can knock ripened seed to the ground. Seed on the soil surface could not

be collected with the harvesting heads, which significantly reduced yields.

A suction device was developed by Lorenzen and Coates (5) to collect the shattered seed from the soil surface. The head, shown in Figure 5, rides along the soil surface on a skid shoe and yet remains free to pivot upward when an obstruction is encountered. The lower end of the head is shaped to conform to the furrow bottom, with rubber belting placed around the opening to provide a flexible seal with the soil surface. This improves efficiency by providing more effective air movement.

Field trials of the device indicated acceptable performance, with large variations recorded in the amounts of seed and trash picked up. Table 2 presents the harvest data for two varieties of guayule in which seed was harvested directly from the plant, and then collected from the furrow.

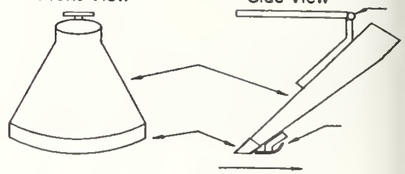

Figure 5. Device designed for collecting seed from the soil surface.

Table 2. Comparison of plant and ground harvesting techniques.

Variety	Mechanical harvesting		Ground harvesting		Yield increase
	Yield (kg/ha)	Germination (%)	Yield (kg/ha)	Germination (%)	(%)
12229	1.42	83	0.56	89	39
N576	2.43	83	1.41	68	58

These data show that not only can significant amounts of seed be collected from the soil surface, but also that the seed is viable and consequently well worth collecting. However, the cost of cleaning the dirt and trash from the seed, along with seed price, would have to be taken into account to determine the full value of ground seed harvesting.

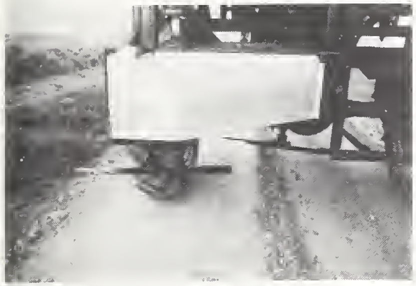

Figure 6. Single-row trimmer for hedging guayule shrub.

Hedging for Seed Production

One of the problems of harvesting guayule seed arises because seed is produced over the entire surface of the plant. This makes it virtually impossible for any mechanical device to contact all of the seed, which consequently limits harvester efficiency. Mechanical shaping of the plants has been considered as a solution since this technique has been successful in other crops, either for improving yields or for bringing the fruiting part of the plant to the surface to assist in harvesting.

Trimming both the sides and top of guayule rows is mechanically possible, but would necessitate the design of a complicated device requiring more power to operate. Limiting trimming to the top surface of the plants would greatly reduce the equipment complexity and possibly could result in the majority of the seed being formed on the top of the plants, where it would be readily accessible to a mechanical harvester.

A single-row trimmer, shown in Figure 6, was developed to permit functional evaluation of the equipment as well as to conduct field-scale testing of the trimming concept. The trimmer consists of two 60-mm lawn mower blades, each attached to a vertical drive shaft, with one positioned slightly ahead of the other in order to provide an overlap when cutting. Overall cutting width of the trimmer is 1 m. The blades are powered by a hydraulic motor through a twin V-belt assembly, which increases rotary speed to 2,500 rpm. Shielding above the blades protects the drive assembly from flying debris and directs the cut material to the side, thus preventing it from obscuring the operator's view.

For the field trials, three varieties of guayule (11605, N576, and N565) were hedged twice yearly and at two different heights (6). The times chosen for trimming were during the dormant season, January-February, and during the spring growth period, April-May. The two hedging heights were chosen so as to remove either just the tips of the top branches, or the top third of the plant. The trials were conducted over two growing seasons, with seed from the hedged and

control rows then harvested mechanically. To reduce field variability, pairs of rows were chosen randomly for each of the treatments, with three replications used.

The results of the trials were inconclusive. In 1986 the control plots yielded significantly more seed than did the hedged plots of N576 and N565, but no statistically significant differences were found for 11605. In 1987 no statistically significant differences in the amounts of clean seed harvested were detected among the treatments.

SHRUB HARVESTING

Crop Characteristics

Rubber accumulates as latex in individual cells, with most of it being concentrated in the bark of the roots, stems, and branches (7). Shrub harvesting for rubber production can take two forms. The plant may be clipped above the crown and have only the branches and stem removed, or it may be undercut 150 to 200 mm below the soil surface and removed with the top of the taproot intact. The former method reduces the expense of ground preparation and replanting after each harvest (1). Hunter (8) reported that cumulative yields of rubber from plants harvested once by clipping at two years of age, followed by digging at the end of the sixth year, were greater than the yields from unclipped plants of comparable age. Garrot (9) found that clipping at two years followed by digging at four years yielded 71 percent more rubber per plant than digging alone. In the clipping trials, plant survival rate was found to be superior for plants clipped at 100 mm compared to those clipped at 50 mm, at ground level, or undercut 50 mm below ground level.

Mature guayule plants can reach a height of 1.3 m and have a main stalk diameter greater than 50 mm. Plant stalks and branches are woody in nature, so cutterbars and rotary devices used for the harvest of most agricultural crops are unsuitable for this application. Additionally,

Figure 7. Shrub cutter fabricated using two plow coulters. A plain coulter is on the left, a notched coulter is on the right.

since the plant can have a relatively large taproot, undercutting systems must be constructed to withstand shearing of the root below the soil surface.

Equipment for Cutting Guayule

In a series of trials conducted by Coates (10), two cutting devices were evaluated. One system consisted of two 0.3-m-diameter circular saw blades, each powered by a hydraulic motor operated at speeds to 5,000 rpm. The second system, shown in Figure 7, was fabricated using two 0.43-m-diameter plow coulters, one notched and the other plain, spaced 0.39 m apart. Powered by hydraulic motors, the coulters were operated at speeds to 160 rpm.

Field and laboratory trials of the cutting devices showed the coulters to outperform the saws. On the average, 2.5 times the energy was required per kilogram of crop harvested for the saws compared to the coulters. Ground speeds of up to 3.2 km/h proved no problem for the coulters, with higher ground speeds being realized throughout the trials for the coulters than for the saws. Functional evaluation showed the coulters to be safer and more durable than the saw blades. When the saws contacted a stone they dulled, whereas the coulters were relatively unaffected. Even without contacting foreign objects, the saws lost their edge rapidly, with three sets of saws required to cut an equivalent area cut by one set of coulters. This rapid wear was a result of the operating environment, which required cutting a sticky-sapped plant material close to the soil surface. The combination of sap and soil, along with the sawed plant material, dulled the teeth rapidly and accumulated on the blades. Neither the dulling nor buildup occurred with the coulters.

Additional advantages of the coulters were their ability to cut down to the soil surface without blade damage, and their improved performance in moist field conditions where small branches, weeds, and grassy materials tended to plug the saws.

Plant Digging Equipment

Requirements for equipment to dig guayule shrub are the ability to undercut the plants at a depth of approximately 150 mm, to lift the plants to the soil surface, and to remove as much soil from the roots as possible.

A machine developed to dig guayule is shown in Figure 8. The two-row, trailed implement is constructed with a single blade inclined at an angle of 30 degrees from horizontal. A hydraulic ram permits adjustment of digging depth and raises the blade for transport. Immediately behind the blade, two lifting chains similar to those used for potatoes, carrots, and other root crops lift the plants and permit the soil to drop through to the ground surface. Beneath the chains a pair of V-shaped blades is mounted on a hydraulically adjusted frame. These blades level the surface by directing soil from the beds into the furrows between them. This provides a smoother surface on which the plants are deposited and assists with later pickup of the shrub. At the rear of the digger are three adjustable deflectors fabricated from 25-mm pipes spaced 100 mm on center. The bottom deflector permits larger clods that have been carried up the lifting chains to fall through, while conveying the plants rearward. Additionally, when the plants drop from the conveying chains, they strike the pipes, thereby removing soil attached to the roots. The two side deflectors can be adjusted to funnel two rows of plant material together if desired. If only a single row is to be

Figure 8. Implement designed to undercut and then lift guayule shrub onto the soil surface.

dug, one deflector can be used to move the plants to the center of the implement, providing more clearance between the dug material and standing plants (11).

In addition to the draft required to move the digger through the soil, power take-off (PTO) power is required to drive the conveying chains. Field tests of the implement showed average PTO and draft power requirements to be approximately 2.6 kw, and 5.3 kw, respectively, when the digger was operated at 2.8 km/h.

Densification Equipment

To facilitate economic transport of guayule shrub from the field to a processing facility, a means of densification is required to minimize handling and transport costs. Such a system must provide the following: a) low energy requirements per unit weight of material densified; b) densities that can optimize capacities of conventional transport vehicles; and c) minimal decreases in rubber quality and quantity during transport and storage.

Typically, densification is achieved either by compression or by chopping. Both methods have the same effect in that they reduce the amount of void space present. In agricultural operations, baling using one of several basic types of balers or chopping using a field chopper are the two most common methods of densification. In cotton-producing areas, cotton module makers are also used to densify the crop and facilitate transport to the gin.

Baling. Balers can be divided into three major categories: small square balers, large square balers, and large round balers (12). Small square balers are manufactured in two bale-chamber sizes (36 x 46 cm and 41 x 46 cm) and are normally adjusted to produce bales ranging in length from 91 to 102 cm. Bale densities in hay vary from 130 to 225 kg/m^3, with baling capacities ranging from 11 to 18 metric tons/h. Large square balers produce bales with approximately equal width and height. Depending on the manufacturer, these dimensions can range from 1.2 to 1.5 m, whereas the length is adjustable and is normally set to approximately 2.5 m. Density for these packages varies considerably, ranging from 100 to 240 kg/m^3. Large round balers

produce bales from 1.2 to 2.1 m in width, which can vary in diameter from 1.5 to 2.1 m. Densities from 100 to 200 kg/m^3 are common when baling hay.

Round balers are constructed using either variable-chamber or fixed-chamber design (Figure 9). In the variable-chamber design, either belts, or slats and chains, roll the material into a cylindrical shape as it enters the baling chamber. As the volume of material increases, the forming belts/chains move outward, keeping a constant pressure on the outside of the bale as it forms. In the fixed-chamber design, a series of powered rollers or belts are arranged around the exterior of the bale chamber. As the material enters the chamber, it tumbles as a result of the contact between it and some of the driven components. When the volume of material in the chamber has grown sufficiently, it makes contact with the entire inner surface of the bale chamber. At this time the pressure on the material increases and densification begins. This produces a bale with a core that is less dense than its exterior, the idea being that it permits drying of the material inside the bale while still making the package relatively weather resistant, since it has a dense outer shell.

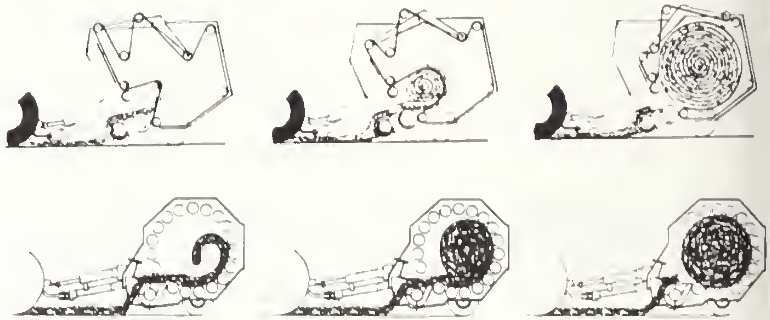

Figure 9. Two types of round balers. A variable-chamber design is shown in the top series of drawings and a fixed-chamber design is shown below.

All three categories of balers have been evaluated for harvesting guayule shrub. Standard small square balers were used in the 1940s to bale guayule (1). Some modifications, however, were required to improve durability and performance. It was reported that this method was adopted over chopping primarily to facilitate shipping the shrub by trucks or rail over long distances without deterioration. In 1987 Coates (13) reported on the use of a small square baler for baling guayule shrub. Although no mechanical problems were noted, stability of the packages was questionable. Hand loading into an enclosed truck proved workable, with most of

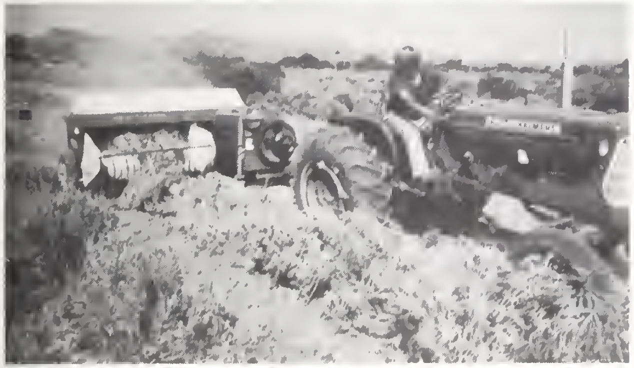
Figure 10. Small square baler operating in a field of guayule.

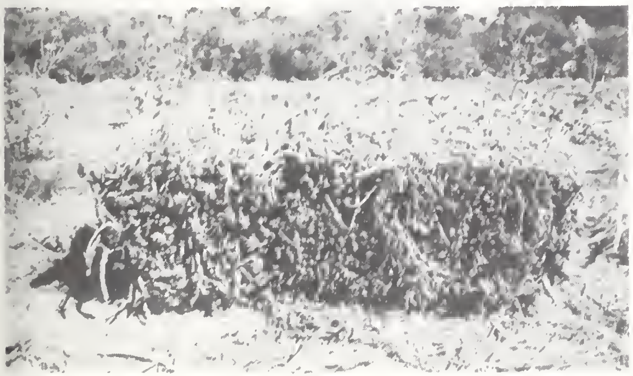
Figure 11. Typical small square bale of guayule shrub.

the bales maintaining their integrity. Firmness of a bale depended to a great extent on the size of material that comprised the majority of shrub in it. If the shrub was mostly large in size, the bales tended to be less stable than if larger material was intermixed with smaller branches and plants. This observation led to the conclusion that unless a field was relatively young, bale instability would preclude the use of most mechanical handling equipment, thus making small square baling impractical for large operations. One additional problem thought to exist, although not experimentally proven, was that a significant reduction in rubber quality and quantity would probably occur with small square balers. This would arise from the shearing of shrub that occurs with each stroke of the baler plunger. This action results in both sides of the bale having cut edges, thereby exposing a large percentage of severed shrub to the air. Exposed surfaces have been shown to be deleterious in terms of rubber quality and quantity. Figure 10 is a photograph of such a baler working in the field, and Figure 11 shows a typical bale produced.

Large square balers have been used in mature crops of guayule. Although some bales have been produced using one manufacturer's baler, the results have generally been disappointing (13). Two major problems with this type of baler appear to exist. The mechanism that feeds the crop from the pickup into the bale chamber has not performed satisfactorily. The design restricts the flow of material, resulting in overloading and frequent breakage of shear bolts designed to protect the baler drive mechanisms. This has reduced baler capacity significantly. A second problem has been the knotters. Consistent tying of all twines did not take place. This was probably aggravated to some extent by the inconsistent

feeding of material into the bale chamber, but mainly was a direct result of material sticking from the chamber into the knotters, which subsequently interfered either with the threading of the twine or with the tying process itself. Figure 12 is a photograph of such a baler working in the field.

Figure 12. Large square baler operating in a field of guayule.

The large square bales that were successfully made were easily handled with a forklift and hence were quite suitable from this standpoint. However, the problems with reliability combined with manufacturer's concerns for durability has led to the abandonment of this type of equipment for guayule harvesting.

Both fixed-chamber and variable-chamber large round balers have proven effective for densifying guayule shrub. Belt as well as slat-and-chain variable-chamber balers were evaluated by Coates and Lorenzen (14). The chain system was found to have a design limitation that prevented successful operation in guayule. Although the problem could be overcome by extensive modification of the mechanical drive, this was not done, as other balers have performed well with either minor or no modifications required.

One belt-type, variable-chamber baler underwent a series of field tests. Numerous bales were made, with overall performance of the baler as well as quality of the bales produced being acceptable. Belt durability, however, could be a problem for this type of baler. Although the structural integrity of the main cords of the belts appeared to be unaffected, the rough surface of the belts, which helps start and keep the bale rolling in the bale chamber, suffered numerous nicks and gouges. Whether or not this would shorten belt life was not determined. Bale weights for this unit ranged from 700 to 870 kg, with average densities of 500 kg/m^3 being recorded.

Two makes and three models of fixed-chamber balers that use steel rollers, rather than belts, to form the bale chamber were evaluated. All of the balers performed satisfactorily, with two machines requiring the addition of shields and crop-gathering wheels to direct the flow of shrub

Figure 13. Two large round balers shown ejecting bales. The baler shown at the top uses a variable chamber and the bottom baler uses a fixed chamber.

into the pickup. Durability was not a problem. The repairs made were determined to be a result of normal wear and tear rather than a result of baling guayule. Width of the bales produced was either 1.2 or 1.5 m, with diameters averaging 1.2 and 1.7 m, respectively. Bales from the smaller implements averaged 454 kg, and bales from the larger unit averaged 950 kg. Bale densities averaged 235 and 280 kg/m^3, respectively. Figure 13 shows two of the balers tested, and Figure 14 is a photograph of two bales made during the test program.

The bale weights and densities reported above are averages obtained over a range of field and operating conditions. Bale weight and density were influenced significantly by the moisture content of the shrub, the ground speed at which the baler was operated, and the size of shrub picked up. For example, in one test, bale weights averaged 500 kg when shrub was baled after drying for only two days and 454 kg after drying four days. This decrease was due not only to the loss of moisture but also to the decrease in the amount of leaves left on the shrub when baled after drying four days. As leaves do not contain rubber, their loss is desirable since it decreases the amount of material that the processing plant must handle. In addition to influencing bale weight, operating speed also affected field capacity. A decrease in field speed increased bale weight and density, thereby reducing transport costs, but also reduced field capacity, which could subject the crop to adverse weather conditions.

Chopping. In the 1930s the Intercontinental Rubber Company used a special heavy-duty field chopper equipped with a pickup attachment to harvest guayule shrub. The chopped material was delivered by blower to trucks, which hauled the material to the mill for processing. This method

was subsequently abandoned by the Emergency Rubber Project because it was unsuitable for hauling shrub long distances either by truck or by rail (1).

In 1988 the Gila River Indian Community, located at Sacaton, Arizona, used a self-propelled forage harvester equipped with a shear-bar-type cutterhead to chop guayule shrub. The front-mounted pickup fed either one or two rows of guayule, which had been planted on 1-m centers, into the harvester. Length-of-cut could be varied by changing the number of knives on the cutterhead in combination with adjusting the speed at which the material was fed into the harvester. From the cutterhead, the material passed through a fan, which conveyed the material up and out a spout directed to either a trailed wagon or to a truck that followed alongside.

Figure 14. Two large round bales of guayule shrub. A bale made with a fixed-chamber baler is on the left, and one made with a variable-chamber baler is on the right.

Operation of the chopper was reported to be generally satisfactory; however, some problems with durability were noted. Although knife life was not specifically recorded, the amount of soil and rock adhering to the shrub roots could significantly affect knife longevity.

Given the short hauling distances from the fields to the processing plant, degradation of the shrub was not considered to be a problem for this particular operation. If, however, fields were located at greater distances from the plant, a serious degradation problem could occur. Another potential disadvantage of chopping could arise during periods of adverse weather. Such a system precludes the storage of large amounts of harvested material; consequently if chopping was suspended for any period of time, the processing plant would also be shut down.

Cotton module builders. An attempt was also made by the Gila River Indian Community to use a cotton module builder to densify guayule shrub. A plywood partition was placed approximately one-third of the way along the length of the module builder to reduce the amount of shrub required for the test. The module builder was loaded by hand and compressed at appropriate intervals.

The module that was produced retained its shape, and it appeared that it would withstand loading by conventional live-bed trailers used to pick up and transport cotton modules. Additional testing of this densification method has not taken place, however, since loading of shrub

into the module builder appears to be a significant problem. Hand loading proved to be very labor intensive and time consuming. No equipment is commercially available that could be used directly or could be readily modified to collect and load shrub. Thus in order to use this method of densification, an implement would need to be designed and fabricated specifically for this operation, which would not be cost effective.

Handling and Transport of Shrub

Large round bales of guayule can be handled with devices that have been used for a number of years for hay bales. Several equipment manufacturers produce implements that can load, transport, and unload three or more bales at a time. Front-end-loader devices and three-point hitch implements that have a pair of arms extending from the tractor attachment point can pass under and slightly to each side of the center of the bale. Raising the device causes the bale to be lifted from the ground and cradled between the arms. A forklift can also be used to lift and carry guayule bales. Here again the forks pass below the bale, with one sitting on either side of the bale center forming a carrying cradle.

Commercial transports generally are limited to 27,000 kg net weight, with a usual rule of thumb being that they can haul approximately 1,500 kg per meter of length. Considering that most closed trailers have a width of 2.5 m and a height of 2.7 m and that either two "pups," each 8.5 m long, or a single trailer, 13.7 m in length, is pulled behind the tractor, densities of 235 and 292 kg/m^3 are required to maximize the payload. If flatbed trailers are used they can be loaded to a height of 3 m. Tandem flatbed trailers are either 7.3 or 9.8 m each in length, and single units are 13.7 m long. Based on maximum net weight allowable, densities of approximately 183 kg/m^3 could be hauled on the largest tandem units, and densities of 262 kg/m^3 would be required for single trailers to optimize transport costs.

Although round bales, when considered individually, have densities greater than those

required to maximize transport weights, when they are stacked together on a truck numerous voids are formed that significantly reduce overall load density. The maximum legal width for hauling, without an escort, is 2.4 m; consequently the only bales that can be hauled efficiently are those produced by the smaller round balers, since the width of each is 1.2 m. If the bales are stacked 2 high, 60 bales can be loaded onto a pair of the largest tandem trailers, and 42 bales can be hauled by a single trailer. Using an average bale weight of 454 kg, the net payloads are 27,000 and 19,000 kg, respectively, or 100 and 70 percent of maximum allowable load.

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Chapter 11

Processing

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A commercially viable process for the production of guayule rubber and value-added coproducts must meet fundamental criteria of production efficiency and product quality. The extent to which rubber and nonrubber extractives are recovered and the extent to which the rubber product can be made to meet established quality standards must both be economically acceptable.

PRODUCT SPECIFICATIONS

Developing a product specification unique to guayule rubber will be realized only after commercial users have had considerable experience with the material in a range of applications. The current national stockpile purchase specification adopted by the Federal Emergency Management Agency (FEMA) (1) is based on that for grade 20 natural rubber (TSR 20) from *Hevea* (2). Grade 20 natural rubber is one of several technically specified natural rubbers. Technically specified rubbers offer processing advantages due to their higher uniformity and somewhat lower viscosity compared with other grades (3). Table 1 provides a comparative summary of the specifications for guayule and grade 20 natural rubbers. The Malaysian Rubber Research and Development Board has published statistics on the properties typically found for grade 20 natural rubber (4). These also are included in Table 1.

Table 1. Natural rubber specifications and performance.

Property	Federal Emergency Management Agency specification rubber—*Parthenium*	American Society for Testing and Materials Specification grade 20	TSR 20[a] performance
Dirt, %	≤ 0.20	≤0.20	0.072±0.032
Ash, %	≤ 1.25	≤1.00	0.35±0.08
Volatile matter, %	≤ 0.80	≤0.80	—
Nitrogen, %	≤ 0.60	≤0.60	0.30±0.05
Copper, %	≤ 0.0008	≤0.0008	—
Manganese, %	≤ 0.002	≤0.0015	—
Acetone extract, %	≤ 4.0	not applicable	not applicable
Wallace plasticity, P_o	≥ 30	≥35	44.3±5.9
Plasticity retention, PRI	≥ 40	≥40	67.1±8.4

[a] Grade 20 Standard Malaysian Rubber (SMR 20).

FEEDSTOCK PREPARATION

Chapter 9 describes the impact of biology, environment, and agronomic management on the yield and quality of rubber contained in the shrub. Once the shrub has been harvested and received by the processor, the processor needs to maintain rubber quality until the rubber is isolated. This will be especially important if variations in shrub harvest and transport scheduling make it necessary to stockpile shrub. The most straightforward approach would be to store

shrub as whole plants, minimizing the exposure of unstabilized rubber to air. However, the sheer volume of biomass needed for processing makes it necessary to store densified plant material (Chapter 10).

Historically, shrub has been baled and stockpiled in the open. Pilot-scale operations have successfully used refrigerated storage of field-dried shrub (5). One alternative method of preservation is to mill the shrub, then form it into briquettes prior to storage (6). Antioxidants can be applied to the briquettes to retard rubber degradation. A less attractive alternative is to store ground shrub as a slurry in an organic solvent such as acetone or a dilute solution of resin in acetone (7, 8). While several days' protection is claimed, the need to agitate the slurry makes large-scale storage impractical.

PROCESSING ALTERNATIVES

Unlike *Hevea*, which bears rubber latex in a system of easily tapped ducts, guayule stores its rubber within the cells of its bark and woody tissue. It is necessary to rupture these cells to gain access to the rubber. Historically, human teeth, pebble mills, hammer mills, and single-disk attrition mills have been used for this purpose. A more effective procedure is to subject coarsely ground plant material to a combination of compressive and shear forces (9). This can be accomplished using differential roll mills or extruders of the types used for oilseed processing. Both the rate and efficiency of rubber extraction are increased.

Three basic approaches have been used to recover rubber and resin from the comminuted shrub: flotation, sequential extraction, and simultaneous extraction. In flotation processing, ground shrub is agitated in a dilute caustic solution. Resinous rubber "worms" form and are skimmed from the surface. The worms are washed with a polar organic solvent to remove resin. Sequential extraction involves initial deresination of the ground shrub with a polar organic solvent. The deresinated shrub is subsequently extracted with a nonpolar solvent to

remove the rubber. In simultaneous extraction, the ground shrub is extracted with a rubber solvent to produce a dilute solution, or miscella, of resin and rubber. A polar solvent is added to coagulate the high-molecular-weight rubber polymer. Several reviews summarize past work on flotation and sequential extraction processing (10-12).

Flotation

Flotation processing was evaluated between 1976 and 1980 at a pilot facility in Saltillo, Mexico, operated by the Centro de Investigación en Química Aplicada (CIQA) (13). The facility was capable of processing about one ton of shrub per day. Batches of wild shrub were parboiled and defoliated. The defoliated shrub was then passed through a Bauer single-disk attrition mill. This coarsely chopped shrub was treated with a dilute caustic solution to effect agglomeration of rubber worms, which were isolated and deresinated with acetone. The crude deresinated rubber was dissolved in hexane to allow addition of antioxidants and filtration to remove dirt. Finally, the rubber product was recovered from the clarified hexane cement by steam desolventization.

Flotation processing is a direct descendant of the earliest method of isolating rubber from guayule: chewing shrub branches to yield an agglomerated mass of resinous rubber (14). While the followers of this particular oral tradition were not concerned with removing the resin, the benefit to rubber quality of deresination has been known for some time (15). Worm deresination is the rate-limiting step in flotation processing. The rate-limiting step of the deresination process itself is the diffusion of solubilized resin components through the solid phase of the rubber particles, rather than diffusion of acetone into the particles (16). Desorption of resin into the solvent is enhanced when the moisture content of the worms is above about 10 weight percent. Lower-molecular-weight resin components are removed at higher rates than are higher-molecular-weight components such as fatty acid triglycerides (17, 18). It is the unsatu-

rated fatty acid portion of the triglycerides that has been implicated as the factor in resin that contributes to rubber degradation (19).

Table 2 summarizes the results of analyses carried out on several bales of the CIQA flotation process rubber used as part of the U.S. government qualification program (20). While the samples have high Mooney viscosities, none meets the FEMA specification for acetone extract (4.0% maximum). These characteristics may reflect inadequate deresination. Nevertheless, the performance of the rubber product was satisfactory, suggesting that the maximum acceptable level for acetone extractables could be somewhat higher.

Other considerations aside, the major weakness of flotation processing is the need to dispose of the large volumes of caustic flotation medium. This aqueous waste stream contains substantial levels of dissolved salts, including various shrub extracts, as well as sodium hydroxide. No direct use in agriculture is possible. Consequently, the effluent would require extensive treatment before reuse or disposal.

Table 2. Properties of flotation process guayule rubber.

	Bale		
Property	1	2	3
Ash, %	0.44	0.66	0.96
Volatile matter, %	0.29	0.45	[a]
Nitrogen, %	[a]	[a]	0.13
Acetone extract, %	5.26	5.53	4.47
Mooney viscosity, ML1+4 (100°C)	96.7	105.0	75.3

[a] Data not available.

Sequential Extraction

Sequential extraction directly addresses the weakness of flotation processing: separating resin from rubber. Ground shrub is deresinated by extraction with a polar solvent, typically acetone (21). Alternatively, the dilute resin solution initially produced can be recycled and used as the extraction medium (22). Rubber is then removed from the resin-free shrub by extraction with a solvent such as hexane. An antioxidant can be added to the solvent to stabilize the rubber from the moment of extraction. Extractions can be carried out by immersion, gravity percolation, or counter-current percolation. A significant benefit of sequential extraction is the improved quality of the resin-free rubber (23).

Sequential extraction has been evaluated on a pilot scale in a semi-batch mode at the USDA Northern Regional Research Center (24). Leaves were removed by passing whole-plant material through a tunnel drier. The defoliated shrub was then chopped and flaked. Batchwise deresination with acetone was carried out by a two-stage immersion and rinse. The heart of the operation was a Kennedy oilseed extractor that allowed continuous, countercurrent extraction of rubber with hexane at 60°C. The unit was operated at a biomass feed rate of 12 kg/h. Rubber recovery was effected by steam stripping the hexane cement. Based on comparative two-stage gravimetric analyses of unextracted shrub and extract streams, rubber recoveries as high as 88 percent were achieved. The quality of the rubber product was not determined.

Figure 1 illustrates a continuous pilot-plant operation employing the USDA process. This proposed design is based on conventional oilseed processing technology. Much of the necessary equipment is commercially available, so that the cost of further process development could be significantly reduced. Since the rubber contained in cultivated guayule does not necessarily meet established product specifications (25), the process would have to be modified in such a way as to allow selective recovery of the high-viscosity rubber fraction. In any extractive process, incomplete desolventization of residual solids is a primary cause of solvent losses.

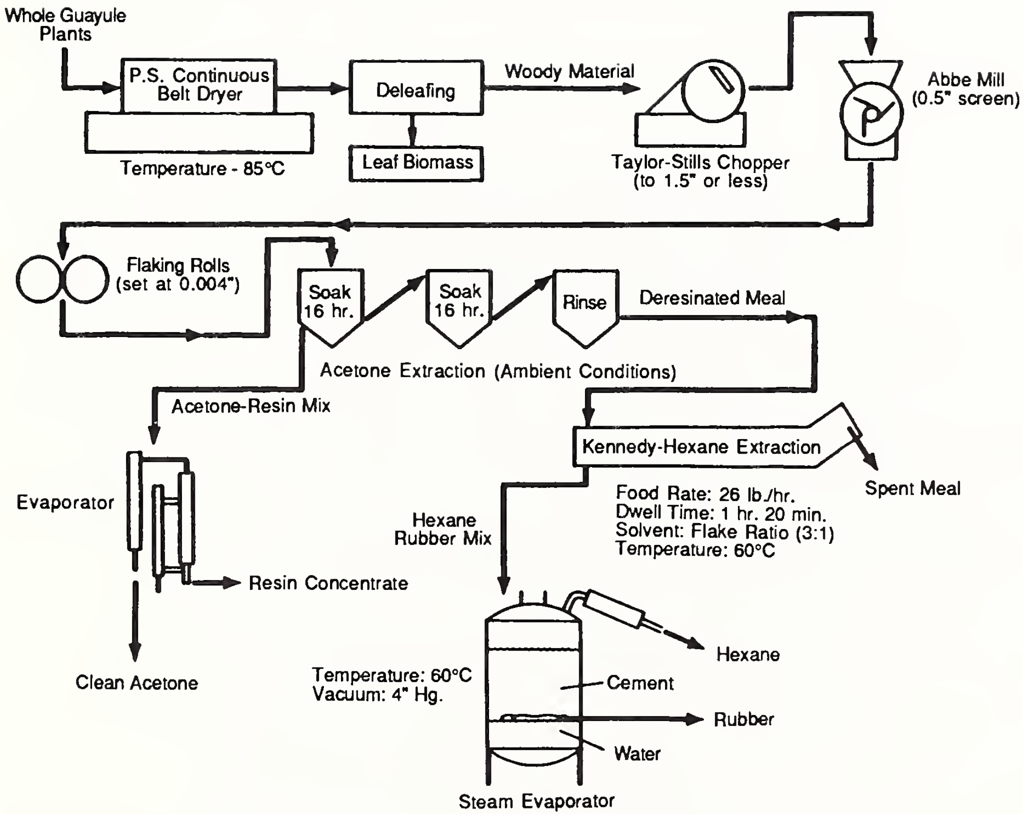

Figure 1. Proposed pilot-plant process using sequential extraction (USDA).

Having two extraction steps, a sequential process would be more prone to solvent loss. Furthermore, the rubber solvent could become contaminated with the resin solvent, complicating its recycle (26).

Simultaneous Extraction

The choice of extraction solvent is particularly crucial in simultaneous extraction. The solvent must efficiently extract both rubber and resin, yet easily yield the rubber upon addition of a polar "rubber nonsolvent." During the rubber recovery step, the rubber/resin solvent and rubber nonsolvent come into contact. Consequently, the ability to efficiently recover and separate the solvent/nonsolvent system components is very important. Solvent toxicity must also be considered. Several solvent systems have been evaluated. Halogenated solvents such as methylene chloride, 1,1,1-trichloroethane, and perchloroethylene can serve as rubber/resin solvents, with methanol and ethanol being used as the nonsolvent component (26). Toluene has been used as the rubber/resin solvent and methanol as the nonsolvent component in one pilot-scale evaluation (5). With these systems, solvent recovery is complicated by the need to add water in order to prevent the formation of undesirable azeotropic mixtures. Replacing toluene with xylene avoids the formation of azeotropes, but bagasse and rubber desolventization is more difficult due to xylene's high boiling point (5). Other systems include the use of a mixture of hexane or pentane and acetone as the rubber/resin solvent and acetone as the nonsolvent (27, 28). One example of this type of mixed solvent is the pentane-acetone azeotrope (78 weight % pentane and 22 weight % acetone). Separation and recovery after rubber coagulation is simplified by the fact that the highly volatile azeotrope (bp 32°C) distills ahead of the excess acetone added to coagulate the rubber. The volatility of the pentane-acetone azeotrope is also a disadvantage. Extraction at temperatures above the low boiling point must be run at pressures above 1 atm. Extensive precautions must be taken to minimize solvent loss.

One means of improving solvent-use efficiency is to extract shrub using recycled rubber-resin miscella (29). Since a single-pass extraction produces a relatively dilute miscella, up to about 90 percent of the miscella can be recycled on a continuous basis. The extent of recycling is limited by the extent to which the liquid phase can be expressed from the plant material and by the viscosity characteristics of the recovered liquid phase. Liquid phase expression can be carried out by means of a centrifuge, screw press, or extruder. Fresh solvent is used only to rinse the bagasse and to replenish that portion of the miscella not recycled. Final separation of bagasse and miscella can be effected by the addition of water (30). A two-phase system forms, with the water-logged bagasse located just below the interface. The pentane-acetone azeotrope is the preferred solvent for this application. However, processing is complicated by the need to recover substantial quantities of acetone from the aqueous phase.

A key advantage of simultaneous extraction is the capability of tailoring the physical properties of the rubber product. The rubber recovery step is a coagulation involving the addition of a polar organic solvent to a rubber cement, the rubber-resin miscella. As in any coagulation, the highest-molecular-weight polymer fraction is precipitated first. By an appropriate choice of the number of fractionation stages and the temperature at which the fractionation is carried out, the bulk physical properties and resin content of the rubber can be adjusted to meet product specifications (31). This capability is most important in light of the variable nature of the rubber quality noted in some shrub lines (25).

Past the point where product rubber is produced, coagulation can be continued to isolate the balance of the low-molecular-weight polyisoprene as a processing coproduct. Under these conditions, the fractionator effluent consists of a dilute solution of resin. Once desolventized, the resin itself can be fractionated by mixing with a hydrocarbon solvent, such as pentane, in the presence of a small amount of water (32). The resulting solution of the less polar resin components can be decanted from insoluble polar material. The incentives for such additional processing are discussed in Chapter 12.

CASE HISTORIES

What follows are case histories of two recent process development programs carried out by Texas A&M University and Bridgestone/Firestone, Inc. (formerly The Firestone Tire and Rubber Company). Both programs represent applications of simultaneous-extraction technology.

Texas A&M University Extraction Processes

Two pilot-scale (1 ton/day fresh-shrub basis) simultaneous-extraction processes were developed at Texas A&M University (5, 33, 34). The first process is based on a batch-mixing screw-pressing extraction step (33). The second process illustrated in the flow diagram in Figure 2 is similar to the first process except that the batch-mixing screw-pressing step was replaced with a continuous countercurrent extraction step (5). This step utilizes a Crown shallow-bed extractor operated in a nonconventional dilute miscella mode. Both processes are compatible with the basic infrastructure of cottonseed oil mills located in the southwestern United States. The principal difference for guayule processing is that distillation is required to separate the solvents while simple steam stripping is needed to separate the solvent (hexane) from the nonvolatile cottonseed oil fraction. The major cost to retrofit a solvent-based cottonseed oil plant is, therefore, expected to be in the downstream solvent recovery section. The principal equipment is, however, readily available as "turnkey" systems from engineering construction firms engaged in petrochemical or petroleum plant processes.

Between mid-1984 and late-1986 the Food Protein Research and Development Center at Texas A&M evaluated the effects of different shrub types, preparation methods, different solvents and changes in operating parameters on rubber yield. The downstream operations involved recovery of: guayule resins, other acetone solubles present in the leaves, rubber, and

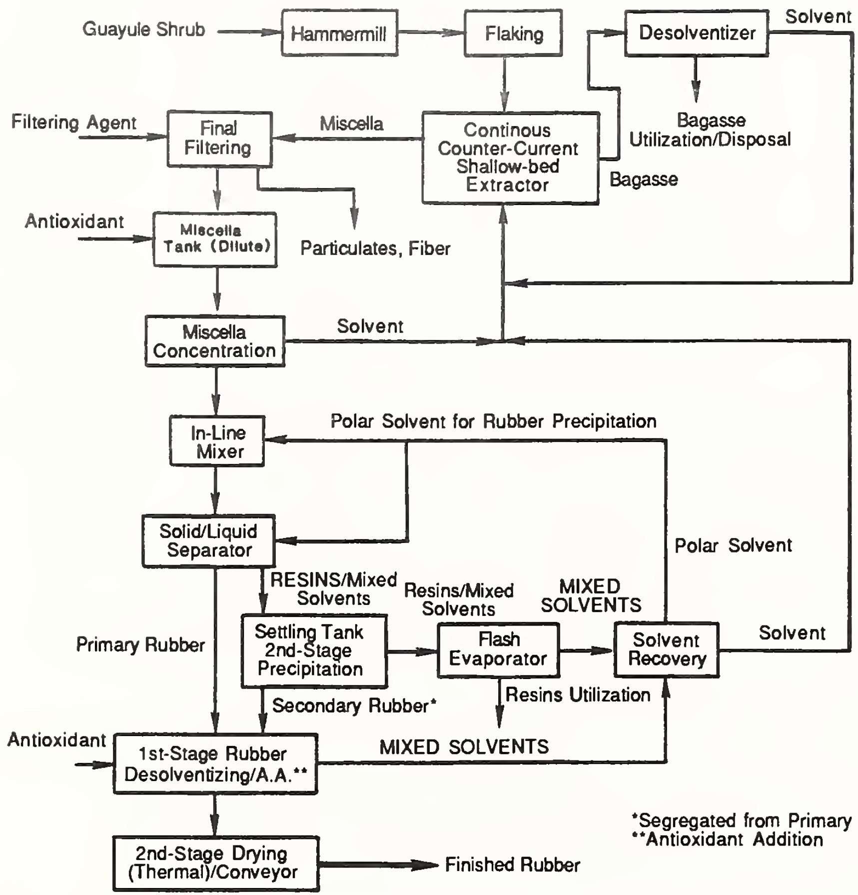

Figure 2. Continuous simultaneous solvent extraction process for guayule (Texas A&M).

alcoholic solvents. Approximately 600 pounds of rubber were prepared on an intermittent plant-operating basis over a total time period of operation of nearly four months. The majority of the rubber (approximately 400 pounds) was prepared with xylene and toluene as solvents, with a lesser amount prepared with a blend of pentane/perchloroethylene. Compounding studies, physical testing according to American Society for Testing Materials (ASTM) standards, molecular-weight determinations and product-evaluation tests were used to assess the adequacy of the Texas A&M extraction processes. Rubber yields up to 92 percent of the available rubber in the shrub (as per Soxhlet analysis) were obtained with single-pass flaking. Based on these results and continuous operation of the plant using recycled solvents and methanol, the overall operation of the Texas A&M continuous process was judged to be a success.

Shrub feedstock and preparation procedures. Various USDA shrub lines were evaluated. These included shrubs designated as native, California bulk, dryland and improved varieties. The improved varieties included shrub designations N565, N567, 11591, 11605, 11619, and 12229. The largest single fraction processed, shrub 11605, came from Texas Agricultural Experiment Station plantings in Pecos, Texas. Baled clippings from USDA plantings in Brawley, California, and whole shrubs from USDA in Weslaco, Texas, were also evaluated. All plants were air dried in the field prior to shipping and upon delivery were kept under refrigeration at around 5°C. No efforts were expended in removing the leaves since this was considered impractical. All shrubs were processed within approximately eight months after receipt of shipment. The shrub age varied from around two to four years.

Shrub preparation prior to extraction consisted of first removing the chilled whole shrub from refrigeration either the afternoon preceding or early morning of the same day. The shrub was then hammer milled and flaked in a single pass for the continuous process and occasionally flaked for the screw-press operation. Although multiple flaking was expected to improve extraction yields due to more complete cell rupturing, this was not investigated because of lack

of a double-roll flaker and time limitations. The flaked shrub was then extracted within a one- to two-hour time period. The average shrub moisture content was 15 percent by weight. It should be emphasized that this moisture content is the equilibrium moisture content resulting from storage under refrigeration.

Results and Discussion

Toluene, xylene, perchloroethylene (PERC), pentane, hexane, and blends of PERC with pentane and hexane were the solvents evaluated over a temperature range of 100 to 125°F. Methyl, ethyl and isopropyl alcohol were the polar precipitants tested over a 60 to 100°F temperature range.

Batch pilot plant evaluation. Table 3 presents selected processing data for different shrubs, solvents, alcohols, and antioxidant treatments. Noticeable differences in rubber quality were obtained between the Brawley, California, and the Pecos, Texas, shrubs. The Brawley rubber was quite soft and tacky after desolventizing, in contrast to the rigid nonsticky rubber samples prepared from the other shrub types. Rubber yield increased with the number of extractions: two recycles were considered optimum. Although flaking hammer-milled shrub prior to screw-pressing/extraction operations seemed to increase rubber yield, the data were not conclusive because of difficulties in obtaining accurate mass balances. No significant differences in rubber yields were observed between the different solvent types and alcohol precipitants tested. Of the two antioxidants examined, Butyl Zimate and Agerite Resin D, Butyl Zimate was unacceptable since it acted as an accelerator in compounding studies.

Product-quality comparisons of Texas A&M rubber samples are made with Mexican guayule and SMR-20 (*Hevea*) rubber in Table 4. The data indicate that the ASTM D-2227 requirements for contaminants and chemical species can easily be surpassed. Although the initial plasticity values, P_o, are up to 32 percent lower than the ASTM requirement, the Wallace

Table 3. Guayule rubber sample production data.

Processing variable				
Shrub source	Brawley, CA	Pecos, TX	Pecos	Pecos
Variety	mixed	mixed	11605	11605
Harvest	clippings mixed	whole bale	whole bale	whole bale
Post-harvest storage	3.5 mo. 5°C	7.5 mo. 5°C	1.3 wk. 5°C	2 wk. 5°C
Ground shrub holding time	<4 h	<4 h	<1 h	<1 h
Extraction conditions				
Solvent	PERC	PERC/hexane	toluene	toluene
No. extractions	1	3	2	2
Total time	30 min.	40-50 min.	45 min.	45 min.
Temperature	ambient	ambient	162-168°F	170°F
Miscella holding time	24 h	24 h	<4 h	<4 h
Precipitation conditions				
Solvent	ethanol	ethanol	IPA[a]	methanol
Antioxidant conditions				
Type	Butyl Zimate	ARD/BZ[b]	ARD	ARD

[a] IPA = isopropyl alcohol.
[b] ARD = Agerite Resin D; BZ = Butyl Zimate.

Plasticity Retention Index (PRI) values are double the specification. Product-quality comparisons are considered favorable for the Texas A&M samples considering that they were prepared during initial plant "debugging" operations in mid-1984.

Continuous pilot plant evaluation. The continuous extraction process illustrated in the

Table 4. Comparison of Texas A&M rubber with Mexican guayule and SMR-20.

Analysis variable	Requirement ASTM-D2227	SMR-20 sample 1	Mexican	A&M samples 1	A&M samples 2
Dirt content (%)	0.2	-	0.02	0.05	0.034
Ash content (%)	1.0	-	0.78	0.57	0.53
Volatile matter (%)	0.8	-	NR[a]	ND[b]	ND
Nitrogen content (%)	0.25 to 0.6	-	NR	0.003	ND
Copper content (%)	0.0008	-	0.00055	0.0002	0.0002
Manganese content(%)	0.00015	-	0.00047	0.0007	0.0003
Acetone extract (%)	4.0	-	3.54	0.91	2.24
Wallace plasticity					
P_o	35.0	-	40.9	27.2	23.9
PRI	40.0	-	89.2	78.7	80.1
Molecular weight					
$\overline{M}_n$	-	200,330	231,170	328,400	230,735
$\overline{M}_w$	-	900,120	1,053,620	813,030	715,655
$\overline{M}_w/\overline{M}_n$	-	4.5	4.55	2.48	2.64

[a] NR = not reported.
[b] ND = not detected.

Figure 2 flow diagram was used to extract rubber from hammer-milled and flaked (single-pass) nondefoliated shrubs. Toluene, xylene, pentane, and blends of pentane with PERC as solvents with methyl alcohol as precipitant were evaluated at different extraction and precipitating temperatures. Solvent to shrub ratios for successful operation of the Crown continuous extractor ranged from 4.0:1 to 6.0:1 (Table 5). Xylene and toluene performed comparably in the

Table 5. Selected guayule extraction data (xylene, toluene, pentane/perchloroethylene).

Shrub	Shrub feed rate (lb/h)	Solvent feed rate (lb/h)[a]	Solvent to shrub wt. ratio	Temp. (°F)	Yield (lb)
Pecos, 11605	75-85	480[b]	5.8:1	125	10.0
Variety	75-85	480	6.0:1	125	7.0
Cal Bulk	75-85	330	5.3:1	125	8.6
Native	75-85	330	4.1:1	100	8.5
Native	75-85	480	6.0:1	125	8.6
Cal Bulk	75-90	480	6.0:1	125	13.1
Variety	75-90	480	6.0:1	125	20.8
Variety	75-90	480[c]	6.0:1	125	10.6
Variety	75-90	480[c]	5.6:1	100	19.9
Pecos, 11605	75-90	480[c]	5.6:1	125	15.7
Pecos, 11605	75-90	480[d]	6.4:1	125	4.6

[a] Solvent is xylene unless otherwise indicated.
[b] Toluene.
[c] Blend of perchloroethylene and pentane. Very difficult to extract; severe filtering problems.
[d] Severe clogging encountered in extractor from use of perchloroethylene.

extraction testing and both solvents produced high-quality rubber. Pentane/PERC blends led to poor bed-percolation characteristics due to guayule fines that floated on top of the bed in the dense mixture. Extraction with 100 percent pentane was not encouraging. Rubber yields appeared to decline and a higher fraction of resinous or leafy extract was retained in the rubber judging by an increase in greenish color. Extraction with 100 percent PERC led to severe extractor clogging problems. Rubber recovery rates as high as 92 percent of the rubber con-

tained in the shrub as per Soxhlet analysis have been achieved. This is a significant improvement over the maximum recovery rates of approximately 40 percent achieved during batch processing.

Solvent recovery operations were demonstrated in that recycled solvents and alcohols produced rubber of comparable quality to the virgin materials. Since xylene does not form an azeotropic mixture with methanol (also with isopropanol and ethanol), its downstream recovery by distillation is less costly than for toluene. With toluene, addition of water is required to effect a phase separation with methanol. This intentionally added water, with a high heat of vaporization, must then be separated from the methanol by distillation at a considerable expenditure of energy. Because of the need for simplicity in recycling solvents with a recovery system designed for another process, the xylene/methanol system was selected for continuous rubber production. Xylene losses due to inadequate desolventizing of the guayule bagasse in a Crown desolventizer (designed to desolventize hexane that boils at 156°F) were anticipated because of the high boiling point of xylene, 282°F. This is not considered detrimental to the selection of xylene; however, more rigorous and, thus, more energy-consuming operations would be required, such as, the employment of a "Dowtherm"-type fluid to effect high-temperature desolventizing. Some difficulties might also be experienced in desolventizing rubber extracted with xylene because of the more severe conditions required for xylene evaporation in contrast to a lower-boiling-point solvent. If future tests present such difficulties, it should be emphasized that the Texas A&M process is not limited to the xylene/methanol pair. Toluene or hexane, in conjunction with methanol, would also serve as viable solvents, but downstream recovery operations would require separating azeotropic mixtures.

Physical testing primarily on toluene- and xylene-extracted samples, selected molecular-weight analyses and selected tests on formulated products carried out at U.S. Army Laboratories or by the U.S. Army Tank Command have given satisfactory results that are indicative of high rubber quality. Weight-average molecular weights, $\overline{M}_w$, up to as high as 1.25 million, have been obtained. Tensile strengths above 4,150 psi have also been achieved.

Bridgestone/Firestone

The Bridgestone/Firestone process is a continuous operation employing simultaneous extraction with the pentane-acetone azeotrope. The need to fractionate the crude rubber product led to the adoption of the acronym SERF (simultaneous extraction and rubber fractionation) to more fully describe the process. Between 1984 and 1987, Bridgestone/Firestone carried out pilot-scale evaluations of the various processing steps. These evaluations were primarily batch operations at each state, with some continuous runs in the areas of extraction and bagasse-miscella separation. A prototype facility with a biomass feed rate capacity of 990 kg (fresh weight)/h was designed and constructed by Dravo Engineering Companies. Located on the Gila River Indian Reservation, this facility began limited operations under start-up conditions in June 1988. The initial period of operation was scheduled to extend through September 1990. Figure 3 provides a flow diagram of the Bridgestone/Firestone SERF process.

Feedstock. The primary feedstock for the operation was the lush, large-biomass "Gila" variety developed by Amerind Agrotech Laboratories and maintained on the land of the Gila River Indian Reservation. The bulk of this shrub was three to five years old at operational start up. Secondary shrub supplies, consisting of various USDA lines, came from various sites in the southwestern United States. This shrub was five years old or older. Table 6 summarizes shrub compositions, rubber quality, and estimated yield of usable rubber from these sites as of early 1988. The term "usable rubber" refers to the estimated content of specification-grade rubber in the feedstock. This estimate, based on the Mooney viscosity of the unfractionated rubber, reflects the expected recovery of rubber product after fractionation. A minimum Mooney viscosity of about 72-75 is required to meet a specification for Wallace plasticity of 35 (25). The greater part of the shrub available for processing contained low levels of usable rubber, making it necessary to process larger quantities of biomass to produce a given amount of rubber product.

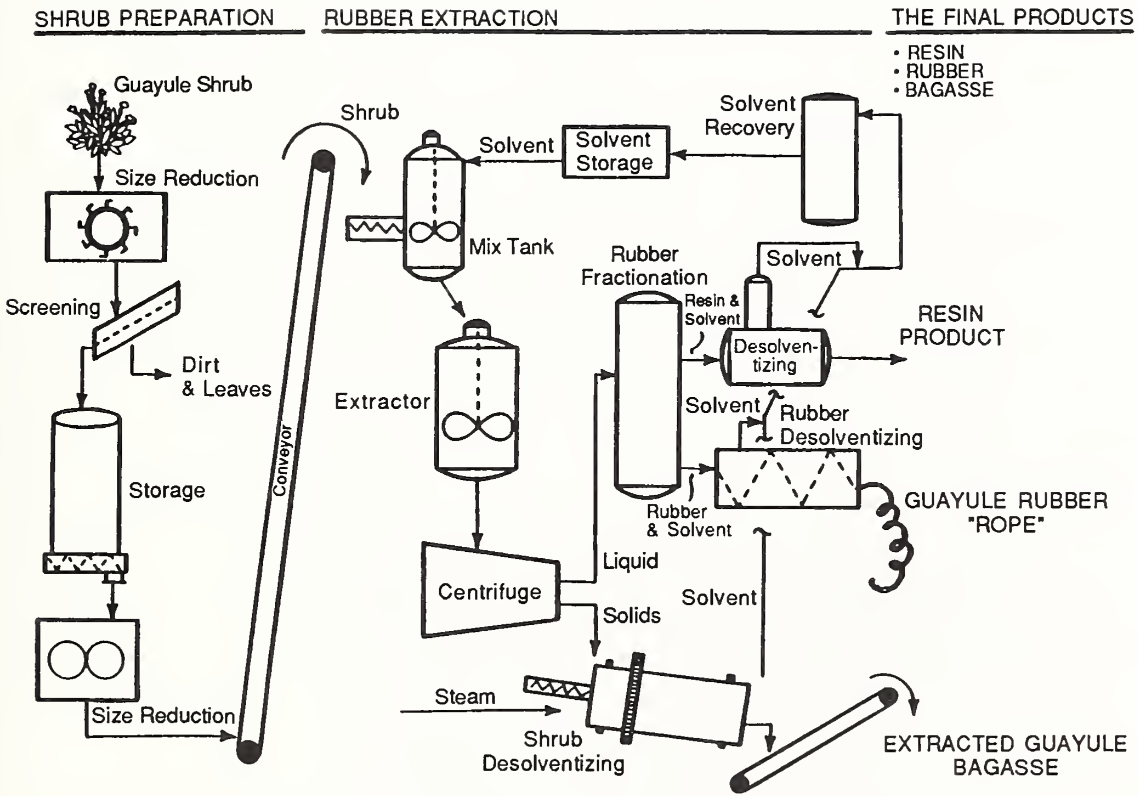

Figure 3. Continuous simultaneous extraction and rubber fractionation (SERF) process (Bridgestone/Firestone).

Table 6. Characterization of feedstock shrub for the Bridgestone/Firestone SERF[a] process facility.

Cultivation site	Resin (%)	Rubber (%)	Rubber Mooney viscosity[b]	Usable rubber (%)[c]
Gila variety				
Sacaton, AZ	8.2	3.5	40.5	1.4
USDA varieties (site composites)				
Las Cruces, NM	8.1	6.8	65.5	5.8
Marana, AZ	6.5	5.8	51.0	3.4
Sacaton, AZ	6.2	6.1	49.5	3.4
Salinas, CA	8.9	8.2	62.5	6.5
Weslaco, TX	9.7	8.6	34.5	2.3

[a] Simultaneous extraction and rubber fractionation.
[b] Resin content of rubber: 4.0%.
[c] Usable ruber % = rubber % X [(0.019 X Mooney viscosity) - 0.39].

Shrub preparation. While the facility was designed to handle baled shrub, some of the guayule initially processed was chopped in the field. Final size reduction was carried out using a Champion Chop-N-Throw mill. The chopped shrub was passed through a screener or air classifier to remove dirt and a portion of the leaves. Finally, the shrub was passed through a Roskamp two-stage flaking mill to rupture the rubber-bearing cells.

Extraction and miscella separation. The primary extraction "solvent" was recycled rubber-resin miscella. Shrub and solvent were first combined in a premix tank to form a slurry. A hindered amine antioxidant (Santoflex 134) was added at this time. The slurry was then fed into a pressurized, agitated tank for extraction at 50°C. Separation of bagasse from miscella

was carried out in a Bird continuous screen-bowl centrifuge. This allowed solvent washing of bagasse solids to improve rubber and resin recovery.

The only water entering the process was introduced with the shrub. Water is nearly totally insoluble in the pentane-acetone azeotrope, so that all water remained with the bagasse. The desolventized bagasse was landfilled.

Product and coproduct recovery. After final clarification, the solution of rubber and resin in pentane-acetone azeotrope was mixed with additional acetone to coagulate a portion of the rubber. Varying the ratio of acetone to miscella allowed control of the "depth" of the fractionation cut. The number of fractionation stages necessary to produce the maximum yield of specification-grade rubber product was predicated in part on the quality of the rubber in the shrub feedstock. The coagulated rubber was a continuous, viscous fluid phase. Most of the antioxidant added upstream had partitioned into the fractionator overheads phase so that additional Santoflex 134 was added to the swollen rubber prior to desolventization. Finally, the swollen, stabilized rubber was fed into a Baker-Perkins Polycon extruder-dryer for desolventization, then compressed into bales.

The fractionator overheads consisted of a solution of low-molecular-weight rubber and resin in a non-azeotropic mixture of pentane and acetone. Since the operation made no provision for resin separation, the overheads were desolventized and the residue, containing about 20-40 weight percent low-molecular-weight rubber, was stored in drums. The recovered solvent was fractionated to separate pentane-acetone azeotrope from the excess acetone.

Product quality. At the time this manuscript was prepared, operational experience was limited to production of the lot of rubber product. Table 7 summarizes the physical properties of several batches from this lot. Overall, the rubber product meets existing specifications for grade 20 natural rubber. Performance testing in compounded stocks will be necessary to establish the commercial acceptability of this material.

Table 7. Properties of guayule rubber from the Bridgestone/Firestone SERF[a] process.

Property	Performance[b]
Dirt, %	0.01±0.00
Ash, %	0.5±0.1
Volatile matter, %	0.6±0.2
Copper, %	0.0005±0.0002
Manganese, %	0.0014±0.0004
Acetone extract, %[c]	3.2±0.8
Antioxidant, %	0.9±0.3
Wallace plasticity, P_o	38±3
Plasticity retention, PRI	60±6

[a] Simultaneous extraction and rubber fractionation.
[b] ASTM specifications apply, where appropriate.
[c] Specification: ≤4.0%, excluding antioxidant.

SUMMARY AND CONCLUSIONS

From an engineering standpoint, simultaneous extraction technology appears to be the most acceptable basis for a commercial guayule processing industry. The ability to rapidly extract both rubber and resin, efficiently separate extractives from bagasse, and selectively eliminate low-molecular-weight rubber overcomes some of the weaknesses of alternative approaches. Whether even this methodology will be economically feasible will depend largely on the cost and quality of the shrub feedstock. Recent processing experience with cultivated shrub has reinforced the continuing need to increase the rubber content of guayule to compensate for poorly understood variations in rubber quality.

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Chapter 12

Rubber and Coproduct Utilization

William W. Schloman, Jr., and John P. Wagner

In 1977, an ad hoc advisory panel established by the National Academy of Sciences concluded that commercialization of guayule will require the following: a) the development of a standard rubber product of known properties, and b) the commercial utilization of processing coproducts (1).

As in 1977, achieving these goals remains predicated on understanding the nature of the rubber and nonrubber materials produced in shrub processing (characterization) and applying this understanding to the discovery of enhanced-value uses for the materials (utilization).

RUBBER

Characterization

Guayule is the only temperate-zone plant that produces a linear, high-molecular-weight *cis*-polyisoprene rubber (2, 3). The molecular-weight average and molecular-weight distribution (polydispersity) can vary with cultivar (4) and as a result of water stress and seasonal factors (5). Other *Parthenium* species produce low yields of low-molecular-weight oligomers (6, 7). The progeny of interspecific hybridization inherit this characteristic. Most of the high-molecular-weight rubber in guayule is produced in the bark (8). ^{13}C-NMR has been used to probe the

details of guayule rubber microstructure (9). In addition, the crystallization behavior of guayule rubber has been detailed (10-12).

The extent to which the *cis*-polyisoprene in guayule resembles or differs from that in *Hevea* has been the subject of reviews covering polymer microstructure (13) and rheology (14, 15).

Unlike natural rubber from *Hevea*, guayule rubber contains few reactive substituents such as epoxides, amines (16), or carbonyl groups (17). The nitrogen-bearing substituents in *Hevea* rubber are strongly bound to the polymer chains, possibly in terminal positions (18). These reactive groups support "storage-hardening" reactions that result in the formation of cross-linked polymer gel. Storage hardening competes with chain-scission reactions that lower rubber molecular weight. Guayule rubber is distinguished from *Hevea* rubber by its reduced green strength or unvulcanized stress-strain properties. This difference could be due to the ability of *Hevea* rubber to form cross-links and, ultimately, polymer gel (19).

The linearity of the polymer chain and the low level of gel in guayule rubber influence its rheological properties. Because of its unique structural features, guayule rubber has a lower viscosity and a shorter stress relaxation time (time to irreversible deformation) than does *Hevea* rubber (20). Such differences manifest themselves in the processing characteristics of the polymers.

Utilization

Performance standards for guayule rubber have been established by the Federal Emergency Management Agency (21). These specifications are based on those for grade 20 technically specified natural rubber (TSR 20) from *Hevea* (22). Guayule rubber applications have been the subject of reviews relating to processability (23), compounding and vulcanization (24), and vulcanizate properties (23, 24). These reviews provide extensive comparisons with the performance of compositions incorporating *Hevea* rubber.

The processing performance of guayule rubber is similar to, but significantly different from, that of *Hevea* rubber. Mixing produces a more rapid reduction in the viscosity of guayule rubber than in the viscosity of *Hevea* rubber (25). This allows a more rapid dispersion of fillers and other compounding ingredients, reducing optimum mixing time. The energy consumed in mixing guayule rubber is about 65 to 70 percent of that consumed in mixing *Hevea* rubber.

The sulfur-cure properties of natural rubber compositions can vary with the rubber source. *Hevea* rubber has a higher cure rate (rate of cross-link formation) and attains a higher cure state (level of cross-linking) than does guayule rubber (26). These differences are ascribed to a natural vulcanization activator present only in *Hevea* rubber. Such performance differences as may exist can be minimized by adjusting the cure system used to effect cross-linking (26, 27). Alternatively, the physicochemical properties of the polymer itself can be modified. Adding MNNA, a substituted nitrosoaniline, to guayule rubber during the mixing process increases composition green strength (28). Promoters of this type react directly with the polymer chain, producing a type of cross-link. MNNA also activates cure. The resulting vulcanizates have improved tensile properties. Under accelerated thermal and UV-aging conditions, cured *Hevea* rubber gum stocks show marginally greater stability than similarly formulated guayule rubber stocks (29). The enhanced stability of *Hevea* rubber is ascribed to the presence of naturally occurring antioxidants in the polymer. These differences disappear in formulations containing carbon black as a reinforcing filler.

Residual plant tissues and inorganic matter (dirt) present in guayule rubber can affect its failure properties. The coarser dirt fraction (particle size > 45 μm) is detrimental to both tensile and fatigue to failure properties at levels as low as 0.5 percent (30). Resin, on the other hand, acts primarily as a diluent during cure, reducing the attainable modulus (26).

Guayule rubber has been tested in a range of military applications. In a study of the reinforcement of rubber compositions used in tank track pads, guayule rubber performed in a manner comparable to that of *Hevea* rubber (31). Similarly, the tensile strength, tear strength,

flex fatigue, and heat resistance of natural rubber-based tank track pad recipes, including one incorporating 100 percent guayule rubber, did not vary significantly (32). The U.S. Navy has carried out a partial determination of the feasibility of rebuilding aircraft tires using guayule rubber in place of *Hevea* rubber (33, 34). The rubber was flotation process material produced at the Centro de Investigación en Química Aplicada (CIQA) pilot facility in Saltillo, Mexico. A battery of tests were run on the retreaded tires: destructive testing, dynamometer testing, flight testing, and evaluation in conventional service. The performance capabilities of the guayule rubber were similar to those of *Hevea* rubber. Guayule rubber appears to be acceptable for rebuilding military aircraft tires. Further testing will be required to qualify guayule rubber for government purchase.

Few other direct applications have been reported for guayule rubber. Guayule rubber has been incorporated into a nonstick chewing gum base (35). Compared with other natural rubbers, guayule rubber can be used in a higher-weight percentage of the composition and still provide the desired properties. Finally, blends of guayule rubber with polyolefins such as high-density polyethylene have many of the characteristics of thermoplastic elastomers (36, 37). Their rheological properties make them suitable for extrusion processing or injection molding.

Chemical modification has been used to yield specialty elastomers based on guayule rubber. As another approach to the production of thermoplastic elastomers, guayule rubber can be oxidatively cleaved to produce a bifunctional initiator for the preparation of triblock copolymers (38). A poly(methylmethacrylate)-poly(isoprene)-poly(methylmethacrylate) triblock produced in this manner has mechanical properties comparable to those of other thermoplastic elastomers.

COPRODUCTS

Under irrigation conditions, USDA lines produce about 367 kg/ha/y of rubber, 359 kg/ha/y of resin, and 4,545 kg/ha/y of bagasse (39). Fractionation of the rubber to adjust its physical properties would reduce the yield of final product to about 312 kg/ha/y. On this basis, a commercial-scale processing facility with a nameplate capacity of 25,000 t/y of product rubber could be expected to produce about 4,400 t/y of low-molecular-weight rubber, 28,800 t/y of resin, and 364,500 t/y of bagasse (residual biomass) (40). Fully 94 percent of the guayule biomass ends up as a by-product of rubber processing. This scale of production makes use of the term "coproduct," more appropriate as a reference to any nonrubber portion of the shrub. Assessments of guayule commercialization have assumed significant economic credits for coproducts (41, 42). Ultimately, the estimate of a coproduct's economic return will be based on an understanding of its composition and a demonstration of its utility in a particular application.

Characterization

Low-molecular-weight rubber. Low-molecular-weight *cis*-polyisoprene is an unavoidable product of the rubber fraction process step. Consequently, the properties of a particular batch reflect the molecular weight distribution of the shrub rubber prior to fractionation and the extent to which fractionation was carried out. The low-molecular-weight rubber coproduct differs from the conventional rubber "product" primarily in its reduced molecular-weight average and, as a consequence, its lower bulk viscosity (17). The low-molecular-weight rubber contains a higher concentration of reactive carbonyl groups (3 mmol/kg) than does the guayule rubber prior to fractionation (0.6 mmol/kg). While this higher level is equivalent to that found in *Hevea* rubber (2-6 mmol/kg) (43), storage hardening does not occur due to a low level of other reactive groups.

Resin. The levels of several major component classes have been determined (44, 45). However, resin composition varies with shrub line, cultivation site, harvest date, and processing history (45, 46). No single analysis can provide a definitive profile of guayule resin.

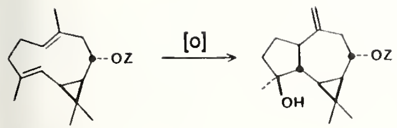
Guayule essential oils are the steam-distillable isoprenoids obtained primarily from leaf resin. The most abundant of the many terpene components are α-pinene, ß-pinene, and terpinolene (47). About 40 percent of the essential oil consists of various sesquiterpenes. In contrast, typical wood and sulfate turpentines contain more than 90 percent terpenes. *Parthenium* species produce various oxygenated terpenoids such as terpinolene and linalool (48). Only guayule and *P. confertum* Gray var. *lyratum* (Gray) Rollins produce bornyl acetate (48, 49). A close or analogous genetic basis is inferred from this.

Epicuticular hydrocarbons from guayule, other *Parthenium* species, and their F_1 hybrids contain C_{19} to C_{40} *n*-alkanes, with nonacosane and hentriacontane as the main components (50, 51). Alkane production is lowest with guayule. Investigators conclude that the variable alkane distribution in whole-leaf waxes is not useful as a taxonomic tool (51).

Guayule epicuticular waxes, primarily found in the leaves and floral parts, contain long-chain alkyl esters of saturated fatty acids, primarily docosanyl eicosanoate (44). Minor levels have been found in guayule seeds (52). The wax is chemically similar to hydrogenated jojoba oil, with physical properties similar to those of carnauba wax. Low overall yield makes it unlikely that the wax would be an economically viable product.

The distribution of the fatty acids present as triglycerides has been determined for woody tissue (45), leaf resins (52), and seed oil (44, 52). In every case, the most abundant component was linoleic acid. No determination has been made of free fatty acids in unsaponified resin. The triglyceride level in whole-shrub resin has been monitored over time (46). Fatty acid triglycerides are most abundant during the winter stress period, with lower levels in succeeding months. As storage substances, accumulated triglycerides are utilized during the period of cold weather dormancy or quiescence.

Perhaps the most distinguishing components of guayule resin are guayulin A and guayulin B (Figure 1), the *trans*-cinnamic acid and *p*-anisic acid esters, respectively, of a sesquiterpene alcohol, partheniol (53). No other *Parthenium* species produces these compounds. The alcohol, a hydroxybicyclogermacrene, is found in other Compositae (54). Guayule has been described as having shifted away from a chemical defense (allelochemical) system based on sesquiterpene lactones to one based on aromatic acids such as *trans*-cinnamic acid (55). Since guayulin production is inherited in hybrids, the presence of these esters can be useful genetic markers for plant breeders (56). The guayulins are easily detected by high performance liquid chromatography (45, 57). The assigned structures have been confirmed by ^{13}C-NMR (58) and X-ray crystallography (59). The greatest concentration of guayulin A has been found in stem and root tissue, the greatest concentration of guayulin B in leaf and root tissue (56). However, whole-shrub levels of both esters showed substantial variation when monitored over a seven-month period (46). The potential for similar variation in distributions within the plant has not been determined.



Parthenol, Z=H
Guayulin A, Z=Cinnamoyl
Guayulin B, Z=p-Anisoyl

8α-Hydroxyspathulenol, Z=H
Guayulin C, Z=Cinnamoyl
Guayulin D, Z=p-Anisoyl

Figure 1. Sesquiterpene esters present in guayule resin.

Of all the many resin components, guayulin A could ultimately have the greatest impact on guayule commercialization. Guayulin A has been identified as a potent elicitor of contact dermatitis in sensitized test animals (60). This allergenicity is comparable in potency to that of parthenin, a sesquiterpene lactone present in *P. hysterophorus* L. Testing in human subjects has not been reported. Nevertheless, the potential for occupational dermatitis as a result of worker exposure to resin during processing has been recognized.

The guayulins are thermally and chemically labile. Heating resin in air increases the proportion of two minor sesquiterpene esters, guayulins C and D (Figure 1) (45). Guayulin C and guayulin D have been identified as the *trans*-cinnamic acid and *p*-anisic acid esters, respectively, of a tricyclic sesquiterpene diol (61). Guayulins A and B are readily converted to C and D under oxidative conditions. The parent diol has itself been isolated from guayule resin (62), as well as the extracts of other Compositae (63).

Triterpenoids, in the form of C_{30} keto alcohols, are major components of guayule resin (45). Structures for five triterpenoids have been confirmed by ^{1}H- and ^{13}C-NMR (Figure 2) (64), refining earlier assignments (65). Argentatins A, B, and C are derivatives of cycloartane. Isoargentatin B (66) and incanilin are derivatives of lanost-8-ene. Two of the triterpenoids have been found in other species: argentatin B in mariola (*P. incanum* H. B. K.) (67), and incanilin in mariola (67) and *P. fruticosum* Less. (66).

Five major flavonoids, methyl ethers of quercetagetin and 6-hydroxykaempferol, and two major kaempferol glycosides have been reported for guayule in a survey of wild stands of North American *Parthenium* species (68). It has been suggested that specific flavonoid profiles in *Parthenium* may be related to particular species (69). Interestingly, the flavonoids apigenin, diosmetin, chrysoeriol, and luteolin 7-methyl ether were isolated from a sample of cultivar 593 tissue (70). None of these flavonoids corresponds to those reported earlier. Methylated flavonoids from guayule are not significantly inhibitory toward larval growth (71).

To date, surveys of resin composition data can account for only a modest fraction of the total resin weight. No single component is present in any great abundance. If the flavonoid profiles are any guide, the compositional details of guayule resin may be more idiosyncratic than once thought. A large-scale processing operation will produce resin from various shrub sources at different times of the year. As a consequence, commercial applications for resin will have to accommodate, or be independent of, varietal or seasonal variations.

Bagasse. Waste biomass from shrub processing will consist primarily of the ground, extracted shrub woody tissue. Other minor components such as leaves and seeds can also be expected to accompany the wood.

Guayule bagasse has a low lignin level (15%) compared with that of commercial wood species (> 20%) (72). The 50 percent holocellulose content is lower than the 60 percent of spruce and maple. Guayule holocellulose is about 40 percent hemicellulose. Taking into account the pentosan in α-cellulose, the overall pentosan content is 27 percent. The pentosans

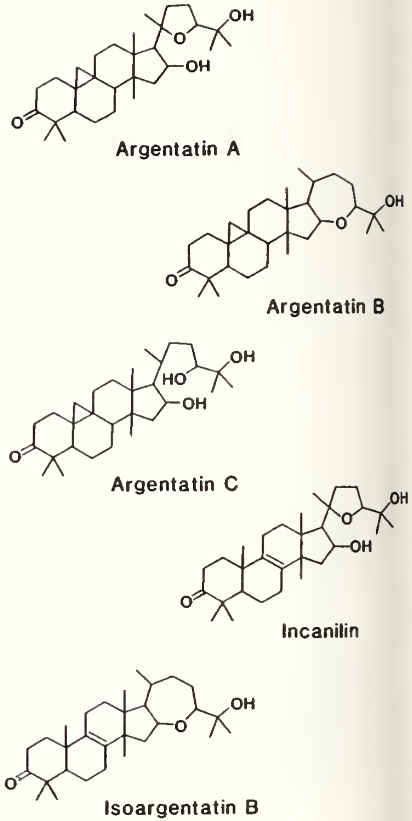


Figure 2. Triterpenoids present in guayule resin.

are exceptional in that the ratio of xylose to arabinose is quite low, less than 3.0 (73). The average holocellulose fiber length of guayule is a very short 0.32 mm.

The residual water-soluble components in guayule bagasse include salts, polyphenolics, and polysaccharides (45). The primary metals detected in the ashed aqueous extract are calcium, sodium, and magnesium. Polyphenolics, defined as components precipitated by lead acetate, represent about 37 percent of the aqueous extract. The polysaccharide fraction, the balance of the aqueous extract, yields mannose and arabinose but not fructose upon dilute acid hydrolysis.

Utilization

Low-molecular-weight rubber. Useful as a plasticizer or processing aid, the low viscosity coproduct polymer can also serve as a feedstock for the production of depolymerized rubber. Depolymerized rubber is a liquid polymer conventionally produced by thermolysis of natural or synthetic polyisoprene. It has wide application in adhesive and molded product manufacture (74). The energy necessary to depolymerize the low-molecular-weight fraction of guayule rubber would be significantly less than that needed to depolymerize *Hevea* rubber. The low-molecular-weight guayule rubber would also be a high-quality supplement to the supply of skim rubber. Skim rubber is recovered from the supernatant liquids produced when *Hevea* latex is concentrated by centrifugation (75). Unlike low-molecular-weight guayule rubber, skim rubber contains various large proportions of nonrubber solids in addition to lower-molecular-weight polyisoprene and must be chemically peptized to improve its processibility.

Resin. Direct utilization of guayule resin has focused on three areas: wood preservatives, arthropod antifeedants, and viscosity modifiers or plasticizers.

Resin shows significant promise as a wood protectant in both marine and terrestrial environments (76). After a one-year exposure in a marine environment, resin-impregnated pine sapwood is only lightly or moderately attacked by arthropod or molluscan borers. After a one-

year exposure in a tropical rain forest, resin-impregnated wood is not attacked by termites. Untreated controls are heavily damaged under these conditions. As important, there is no sign of fungal colonization of the wood. The particular resin component or components responsible for this activity have not been identified. The potential for using resin as a wood preservative for pilings, mine shoring, and telephone poles will increase as creosote comes under greater regulatory pressure.

Having a relatively low average molecular weight, resin can serve as a plasticizer for high polymers. Resin exhibits a high level of compatibility with a typical bisphenol A-based epoxy and diamine cure system. Cured stress-strain properties can be tailored by varying the level of resin incorporation (77). Added to a conventional rubber recipe, the hexane-insoluble fraction of resin improves tack (ply-to-ply adhesion) and green (unvulcanized tensile) strength (78). As important in controlling polymer properties may be resin's chemical composition. Resin acts as a prooxidant, reducing the viscosity of a natural rubber cement in the presence of air (79). Phenolic antioxidants retard this peptizing effect. Studies of guayule rubber degradation have focused on the role of fatty acids derived from resin triglycerides (80-82). Polymer degradation can be directly related to the degree of unsaturation present in the hydrocarbon residue of the acids and, analogously, in the triglycerides actually present in resin (80, 81). By acting as a peptizer, resin can compete with those oxidative processes that lead to polymer cross-linking. Resin actually stabilizes the bulk physical properties of styrene-butadiene rubber (SBR) by retarding the formation of cross-lined gel (83).

Utilization of guayule resin as a chemical process feedstock has focused on the areas of coatings, rubber compound additives, and catalytic conversion to fuels or secondary feedstocks.

Various formulations containing resin have been evaluated in coating applications. With cobalt naphthenate as a drying agent, guayule resin yields a slow-drying coating with good abrasion resistance, but marginal water resistance (79). The physical properties of this particular resin-based coating can be improved by the addition of linseed oil. As an extender or plasticizer in an amine-cured epoxy recipe, guayule resin yields coatings with performance

properties comparable to those of conventional, resin-free formulations (84). Coatings containing 10 percent guayule resin had slightly increased tensile strength. On untreated steel or aluminum, these formulations are strippable. This property is particularly desirable in coatings used for the temporary protection of aircraft or land vehicles.

Modifications that take advantage of resin's diverse composition have yielded additives for improving the performance of rubber compositions. With the proper choice of modifier, chemical derivatization of resin can yield truly resinous (glassy) solids that are much easier to store and handle. Normally, guayule resin is a tacky gum that becomes free-flowing at temperatures above 50°C. Resin or various resin fractions may be treated with formaldehyde, phenol and formaldehyde, or sulfur to yield derivatives that improve the tack and green strength properties of unvulcanized compositions (78). Similar improvements are obtained using resin treated with polyamines or amine-terminated polyethers (85). The sulfurization reaction may involve vulcanization (cross-linking) of the resin triglycerides. Modification with amines or amine-terminated polyethers may involve cross-linking reactions with various keto isoprenoids such as the argentatins. These solid resin derivatives also improve the performance of vulcanized rubber compositions, particularly by increasing tear strength and reducing hysteresis loss. These properties are very desirable in passenger tire applications.

Traditionally, guayule has been given a high rating in surveys of alternative sources of liquid fuels and chemicals (86). These evaluations cite guayule's high yield of nonpolar extractables with high fuel values. Conversion of resin to liquid fuels, as opposed to direct combustion, can be carried out by thermolysis in the presence of a petroleum-cracking catalyst (87). The bulk of the resin is converted to a liquid fraction that includes gasoline and light cycle oil. A significant fraction of the cracking product is noncondensable gas. This indicates that it should be possible to obtain a mostly gaseous fuel under the appropriate conditions.

Bagasse. Guayule bagasse has been considered as a cogeneration fuel, a feedstock for gasification and conversion to liquid hydrocarbons, and a source of fermentable sugars or fiber. These applications are typical of those found for other types of waste lignocellulose.

Preliminary analyses of the products of guayule bagasse combustion, including the identification of potentially toxic species that may form, have been carried out on paraffin-based extruded fireplace logs (88). With a fuel (heating) value of (18,200 kJ/kg, 7,840 Btu/lb), bagasse is an acceptable fuel for direct combustion (89). Although guayule cork has a higher fuel value (26,500 kJ/kg, 11,400 Btu/lb), cork is a unique coproduct of flotation processing and represents a relatively small fraction of the waste biomass. In an application with a value potentially higher than that of direct burning, bagasse can be fed into a fluidized bed gasification system to produce a synthesis gas containing olefins, hydrogen, and carbon monoxide. This synthesis gas can then be catalytically converted into liquid hydrocarbon mixtures equivalent to diesel or aviation fuel (89, 90).

An alternative to the conversion of bagasse to hydrocarbon fuels is conversion to fermentable sugars. Fermentation technology allows the production of a range of oxychemicals including acetic acid, acetone, butanol, methyl ethyl ketone, citric acid, and ethanol. Saccharification by nonenzymatic hydrolysis requires hemicellulose solubilization and disruption of the cellulose crystallite structure. Unfortunately, the level of accessible sugars in guayule bagasse is low compared to other sources of lignocellulose (91). Hydrolysis of the hemicellulose in guayule bagasse requires rather vigorous conditions (73). The low ratio of xylose to arabinose in the hydrolysates means that conversion to xylulose followed by fermentation to ethanol is not practical. With its high pentosan level, guayule bagasse is more attractive as a feedstock for direct production of furfural by acid-catalyzed dehydration. Pretreatment of the residual cellulose with mineral acid or other cellulose solvents followed by dehydration gives low yields of glucose and fructose (73, 91). The resulting mixture is readily fermented to ethanol. Nevertheless, the difficulty of converting guayule bagasse to monosaccharides points out the need for further process development in this area.

The short cellulose fiber length restricts the potential use of bagasse in papermaking (72). Sheets of 100 percent guayule bagasse pulp have reduced strength properties. More attractive

are blends with longer-fibered, bleached kraft softwood pulp. No difficulties were encountered in making sheets from blends with up to 70 percent guayule pulp.

Other guayule-derived biomass. The protein quality of deresinated wood (bagasse), leaves, and seed has been estimated in terms of amino acid content (44). Deoiled (deresinated) seed meal contains between 35 and 40 percent protein. Seed meal can serve as a sole source of protein for mice. In contrast, deresinated leaves contain about 14 percent protein, as does bagasse. Leaf meal is not an acceptable sole source of protein.

SUMMARY AND CONCLUSIONS

The applications demonstrated to date for guayule rubber are essentially the same as those for natural rubber from *Hevea.* Guayule rubber has been shown to be an acceptable substitute for *Hevea* natural rubber. As a consequence, the commercial niche filled by guayule rubber will be defined by the extent to which the product polymer can satisfy existing marketing requirements.

While many uses have been proposed for guayule processing coproducts, the list of applications that have actually been demonstrated is rather short. None of these applications represents an unexpected, high-value use that can be said to result from physical or chemical properties unique to guayule coproducts. This is the particular difficulty associated with complex, multicomponent mixtures such as guayule resin. A coproduct would have no practical application if the material represents too small a fraction of the isolable feedstock biomass (92). In other words, the cost of isolating a material cannot exceed the likely marketing value of the material. For example, fatty acids can be isolated from resin after saponification in yields equivalent to about 166 g/kg of resin (45). Crude tall oil is valued at about $0.16/kg ($0.07/lb). This means that the total cost of resin saponification and fatty acid recovery should be less than about $0.02/kg ($0.01/lb) of resin processed, an unlikely prospect.

The need for large-scale applications testing makes it somewhat difficult to define the size of potential markets with much certainty. However, there are some conventional chemical feedstocks suitable as models for an estimate of coproduct credits. One array of possible coproduct applications is listed in Table 1. This particular set of applications is based on those described in this chapter. Where more than one application is possible for a particular coproduct, such as resin's being used either as a wood preservative (model: creosote at \$0.17/kg, \$0.08/lb) or as a rubber chemical feedstock (models: turpentine at \$0.55/kg, \$0.25/lb; or vegetable oils at \$0.48/kg, \$0.22/lb), the lowest value model was selected for estimation of credit to the processor. Nevertheless, the estimate of total coproduct credit (\$0.75/kg, \$0.33/lb of product rubber) exceeds some estimates of the cost of processing (42). This example clearly illustrates the need for development of higher dollar-valued coproducts. Chapter 13 addresses this issue and shows the viability of such an approach.

Table 1. Coproduct credits based on the estimated output of a 25,000 t/y (product rubber) simultaneous extraction facility.

Coproduct	Production (t/y)		Application	Model	Value (\$MM/y)
Low-MW rubber		4,400	specialty rubber	latex skim	1.7
Resin	28,000	21,000	wood preservative	creosote	5.0
		7,800	rubber chemical feedstock		
Bagasse	364,500	94,900	furfural feedstock	wood chips	12.0
		269,600	energy cogeneration		
				TOTAL:	18.7[a]

[a] Coproduct credit: \$0.75/kg (\$0.33/lb) of product rubber.

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Chapter 13

Recent Advances in Guayule Coproduct Research and Development

Shelby F. Thames and John P. Wagner

INTRODUCTION

The establishment of a guayule-based natural rubber industry will require the commercialization of rubber product(s) of known composition and properties and guayule coproducts. Identification of commercial uses of guayule coproducts is therefore essential. It is well known that the processing of guayule results in the isolation of high-molecular-weight *cis*-polyisoprene (1, 2) and several coproduct fractions. The coproduct fractions include

- a fraction of low-molecular-weight rubber,
- guayule resin,
- a water-soluble fraction of unknown composition, and
- guayule bagasse.

Very recent developments in coproduct utilization demonstrated the potential feasibility for high-dollar-value products. This chapter includes the most recent advances in research and development efforts in:

- refinements in separation and isolation procedures,
- derivation of the low-molecular-weight rubber fraction to chlorinated rubber,
- use of the guayule resin fraction as an adhesion modifier,

- use of guayule bagasse as a hardboard overlay,
- utility of bagasse as fireplace logs, and
- attempted isolation and characterization of a biologically active component of guayule resin.

One of these products, chlorinated rubber derivatives, offers excellent opportunities for commercialization because their current market price is several times that of natural rubber.

RESIN SEPARATION AND PURIFICATION

A critical issue to the development of improved resin coproducts is the separation or purification of the complex resin fraction into various classes of relatively pure compounds. Rubber and Coproduct Utilization (Chapter 12) underscores this need. Although the number of proposed coproduct uses is large, the actual list of demonstrated coproducts is rather short (3). This is due to the difficulty in separating complex multicomponent mixtures, such as guayule resin, in a cost-effective manner. Successful resolution of this problem will facilitate preparation of viable coproducts from the proper chemical species and will eliminate product contamination from various residual resin species. To address this issue, several separation science methods are currently under study at Texas A&M University. This chapter presents selected highlights for membrane ultrafiltration, electrostatic separations, and bagasse utilization research.

EXTRACTION AND PURIFICATION ALTERNATIVES

Figure 1 presents an overview of important separation processes and shows the range of particle or molecular sizes covered by each process and the primary factor governing separation (4). Membrane ultrafiltration techniques appear especially promising for single-step resin

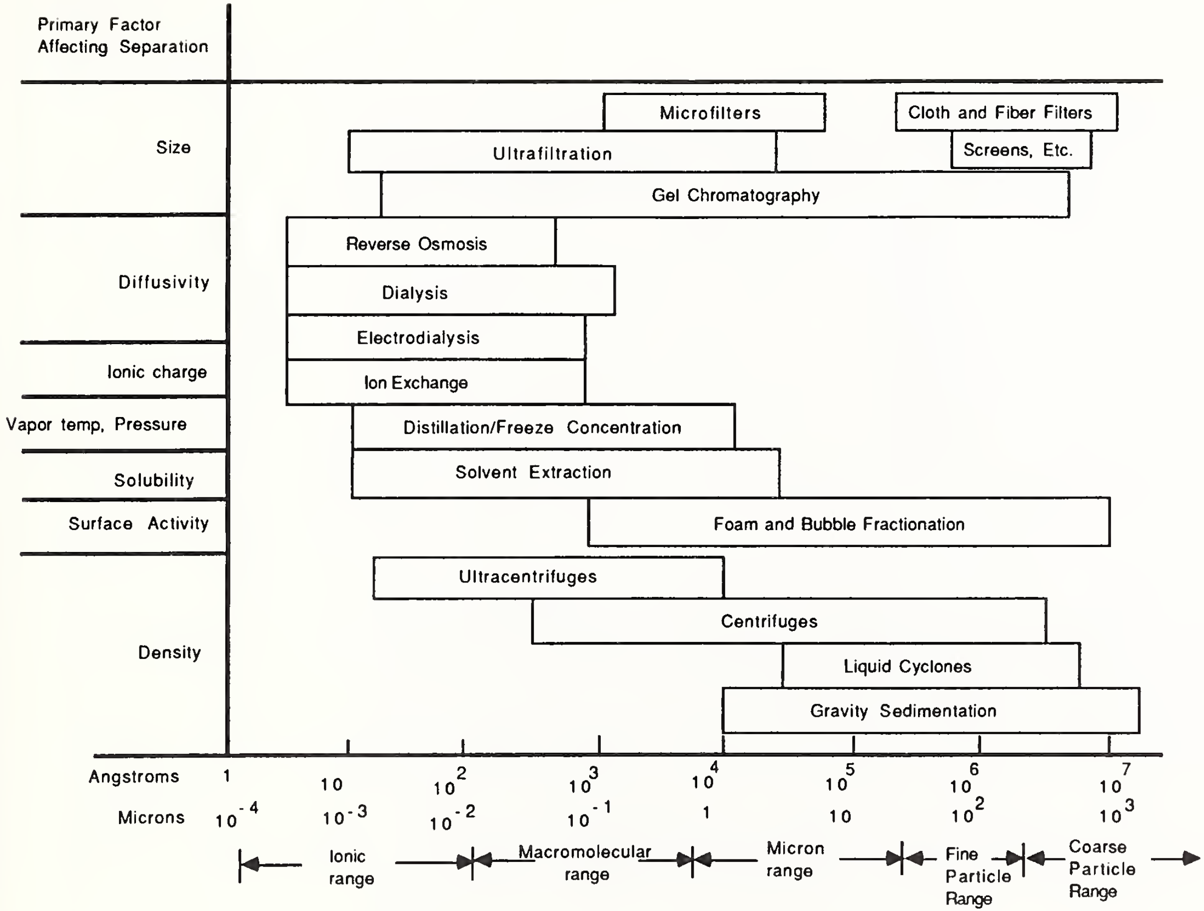

Figure 1. Useful range of separation processes, showing the range of particle or molecular size covered by each process and the primary factor governing each separation process. From Cheryan (2).

Table 1. Characteristics of Amicon ultrafiltration membranes.[a]

Designation	Nominal molecular wt. cutoff	Apparent pore diameter (Å)
UM 05	500	21
UM 2	1,000	24
UM 10	10,000	30
PM 10	10,000	38
PM 30	30,000	47
XM 50	50,000	66
XM 100A	100,000	110
XM 300	300,000	480

[a] Amicon, 1977; Porter, 1979.

purification. Table 1 illustrates the interrelationship of nominal molecular weight cutoff with apparent pore diameter of various Amicon membranes (5, 6). Recent studies at Texas A&M with ceramic membranes (7) have shown that a 65 Angstrom (Å) membrane separated the lower-molecular-weight (MW) components (xylene, MW: 106; and C16—C18 fatty acids such as palmitic, oleic, linoleic, and stearic acids, MW: 256 to 282) from a complex resin mixture with apparent MWs above a few thousand. This approach with a range of ceramic membranes (40, 100, 250, 500, 1,000 Å and larger), as well as with recently acquired inert polyvinylidene fluoride membranes, affords the potential for resin purification in hydrocarbon-based media.

Other separation techniques (Figure 1) for removal of particulates such as microfilters, centrifuges, ultracentrifuges, and liquid cyclones or hydroclones are also under consideration and available in-house. However, because of documented pore clogging associated with microfiltration and high maintenance requirements with centrifuges and ultracentrifuges, lower priority

is given to these techniques. Gravity sedimentation is under investigation as a combined technique with electrostatics.

Commercial applications of electrostatic separations have been in practice for over forty years. However, well-known examples are limited to crude oil desalting, distillate treating (8, 9), and removal of catalyst fines from cat-cracker bottoms (10). Of these examples, crude oil desalting is the most widely practiced in terms of total throughput of product. The process involves blending as much as 10 percent by weight of water with the crude oil to create a fine dispersion, generally with addition of a surfactant, and resolving this mixture under an imposed electric field to transfer salts from the crude oil to the water phase. This approach is potentially applicable to the transfer of salts and aqueous solubles from hydrocarbon-based resins to a second intentionally added component. Water, or another immiscible polar compound, serves as a trial fluid. In addition, particulate matter (e.g., dirt, woody tissue, or other impurities) can also be removed from the resin fraction or from rubber miscellas by electrofiltration.

The applicability of electrostatic techniques to resolve difficult, two- and three-phase emulsions by utilization of high-frequency alternating current (AC) fields has been documented in the patent literature (11-13). Recent data (14) utilizing a model system (i.e., a surfactant stabilized light oil/water emulsion: Isopar M from Exxon; 16.7 percent water by volume and Paranox 100 surfactant from Exxon, 0.5 percent by weight) have shown the potential applicability of this technique to guayule resins and rubber miscellas. Coalescence rates between 25 and 810 ml/min can be achieved between 5 and 15 kV (rms) at frequencies between 60 and 2,000 Hz. Coalescence was not observed for voltages lower than 5 kV (rms).

Because of the high resistivity of typical hydrocarbons used in simultaneous extraction processes, power consumptions are expected to be very low. Current draws are typically in the milliampere range at applied potentials of tens of kilovolts. Current draws are expected to be much greater for processes involving polar compounds, such as methanol, which is used as a precipitant in the Texas A&M process (see Chapter 11, Processing). This is not anticipated to

be a significant deterrent to applicability of this technique. Electrostatic methods apply to much more conductive aqueous type media, and the methodology is referred to broadly as electrodialysis (see Figure l).

The third promising separation technique for resin purification is adsorptive separation. Adsorption processes are used widely in the chemical, biochemical, and petroleum industries, both for purification (removal of trace impurities) and for bulk separations. Although adsorption-purification systems have been in operation for many years for air and water treatment, sugar decolorization, etc., the advent of large-scale adsorption processes for bulk separation is a more recent development. Feasibility was achieved with the development of adsorbents of sufficient selectivity to differentiate chemically similar species.

Zeolites are highly crystalline, hydrated aluminosilicates of ideal crystalline and uniform pore structures. They have been of considerable interest as catalysts for over 20 years because of their high activity and selectivity in various acid-catalyzed reactions. Minimum channel diameters (openings) range from approximately 3 to 10 Å (0.3 to 1.0 nm). This fine pore structure permits only certain molecules to penetrate into the interior and only certain products to escape. In addition to their well-known use as catalysts, zeolites have been used in adsorptive separations. This method is based on differences in molecular size or structure for various compound classes, some of which have similar molecular weights. An example of this method is the separation of n-paraffins from isoparaffins.

About 40 different zeolite framework structures have been discovered, although only a few of these have found commercial application. Examples include zeolites A, X, Y, and XSM-5. Access to the pores of zeolite A is restricted by 8-membered oxygen rings with a free aperture 4.3 Å in the unobstructed Ca^{++} form. These adsorbents are therefore useful for applications involving size-selective adsorption of relatively small molecules.

The pores of zeolites X and Y, which are restricted by 12-membered oxygen rings, are larger than those of zeolite A and have a free aperture of approximately 8.1 Å. These zeolites are therefore useful as adsorbents for relatively large molecules. The frameworks of the X and Y

zeolites are identical; the difference lies in the Si/Al ratio, which controls the cation density and therefore significantly affects the adsorptive properties.

The third commonly-used adsorbent is the intermediate pore (10-membered oxygen ring) zeolite XSM-5, which has a free aperture of approximately 6.0 Å. Zeolite XSM-5 was first prepared by Mobil in the early 1970s. At about the same time a pure silica analog, with the same framework structure, was prepared by Union Carbide and named silicalite. In many adsorbent applications these materials are interchangeable (15).

As a result of the uniformity of the micropores, which are of molecular dimensions, zeolite adsorbents show rather sharp cut-offs with increasing molecular size. This raises the possibility of size-selective molecular sieve separations. Another generic class of adsorbent, bleaching earth, also warrants attention because of its known commercial applicability.

SEPARATIONS AND BAGASSE UTILIZATION RESULTS

Three potentially promising separations methods have been identified in the preceding section for purification of the guayule resin mixture that results from simultaneous solvent extraction processes for natural rubber recovery. In this section highlights of recent results obtained with membrane ultrafiltration and electrostatic separations methods are discussed. Future efforts in adsorptive separations are planned once the limits of applicability of ultrafiltration and electrostatics are established.

Membrane Ultrafiltration Separations

The resin utilized in this research was produced from the Texas A&M simultaneous solvent extraction process illustrated in Chapter 11, Figure 2. The resin is the "bottoms" portion (i.e., higher boiling point compounds) that remains in the flash evaporator after the first solvent recovery step of guayule rubber production. The "bottoms" portion is also rich in the high-

boiling-point solvent, xylene, and contains trace methanol and unprecipitated low-molecular-weight rubber. A comparison of the gross physical and thermal properties of concentrated Texas A&M resins with Bridgestone/Firestone (formerly Firestone Tire and Rubber Company) concentrated resins (by a different simultaneous solvent extraction process in Chapter 11, Figure 3) is given in Table 2. Although both resin samples have essentially identical thermal properties and resemble light oils, significantly higher values are noted for percent carbon residue and American Petroleum Institute (API) gravity measurements for the Texas A&M sample.

Table 2. Comparison of Texas A&M with Bridgestone/Firestone resin properties.

Property	Bridgestone/Firestone resin[a]	Texas A&M resin[b]
Flash point (COC °C)[c]	183	183
Pour point °C	40.6	40.6
BTU/lb.	18,090	18,410
BTU/gal.	154,220	153,275[d]
Carbon residue (%)	0.56	3.52
Sulfur (%)	0.01	0.05
API gravity at 15.6° C	7.08	12.79
Water (%)	trace	trace
Sediment (%)	0.75	1.0
Ash (%)	0.0012	not measured
Specific gravity at 51.7° C	0.998	not measured
Specific gravity at 15.6° C	not measured	0.98

[a] Defoliated shrubs.
[b] Nondefoliated shrubs.
[c] COC - Cleveland Open Cup flash point.
[d] Using specific gravity at 51.7° C.

A schematic of the ultrafiltration apparatus is shown in Figure 2. A hand pump was used to transfer the resin solution from 55-gal resin storage drums to a prefilter vessel. A 25μ-filter removed large particles from the resin solution as it was driven by air pressure from the prefilter vessel to a 10-gal feed tank. After the feed tank was filled, the prefilter vessel and the storage drum were isolated from the system. A 3/4-hp pump transported the solution from the feed tank, through the heat exchanger, to the ultrafiltration unit. The ultrafiltration membranes were ceramic, alpha-alumina, tubular, asymmetric membranes manufactured by Lancy International Inc. (an Alcoa Separations Technology Company). The permeate from the membrane was collected in a graduated cylinder. The retentate passed through a flow meter and returned to the feed tank. The heat exchanger was not used in this process. It was built into the system to process more concentrated resin mixtures that require increased temperatures for viscosity reduction.

At a constant flow rate, data were taken to determine permeate flow as a function of transmembrane pressure difference. The permeate pressure was atmospheric (i.e., zero gauge). Thus, the transmembrane pressure difference is the average pressure inside the membrane. Trial runs were made to determine P_{max}, the pressure difference at which the concentration polarization layer begins to develop. However, because of pump limitations P_{max} could not be determined. Therefore, the operation was always in the linear, pressure-controlled regime.

The results of several trial runs with a 65 Å membrane and analysis by size-exclusion chromatography (SEC) showed that the solvents (xylene and methanol) and the lower-molecular-weight fatty acids were separable in a single step from the resin fraction. Figure 3 details the SEC output signal for the permeate, which shows complete omission of the resin fraction (i.e., the resin fraction was retained as retentate). The details of the analytical methods can be found by consulting Reference 16.

Current efforts under way include testing with a new plate and frame design system,which eliminates the fragile ceramic membranes, and monitoring membrane flux versus pressure drop to provide scale-up data for pilot plant evaluations.

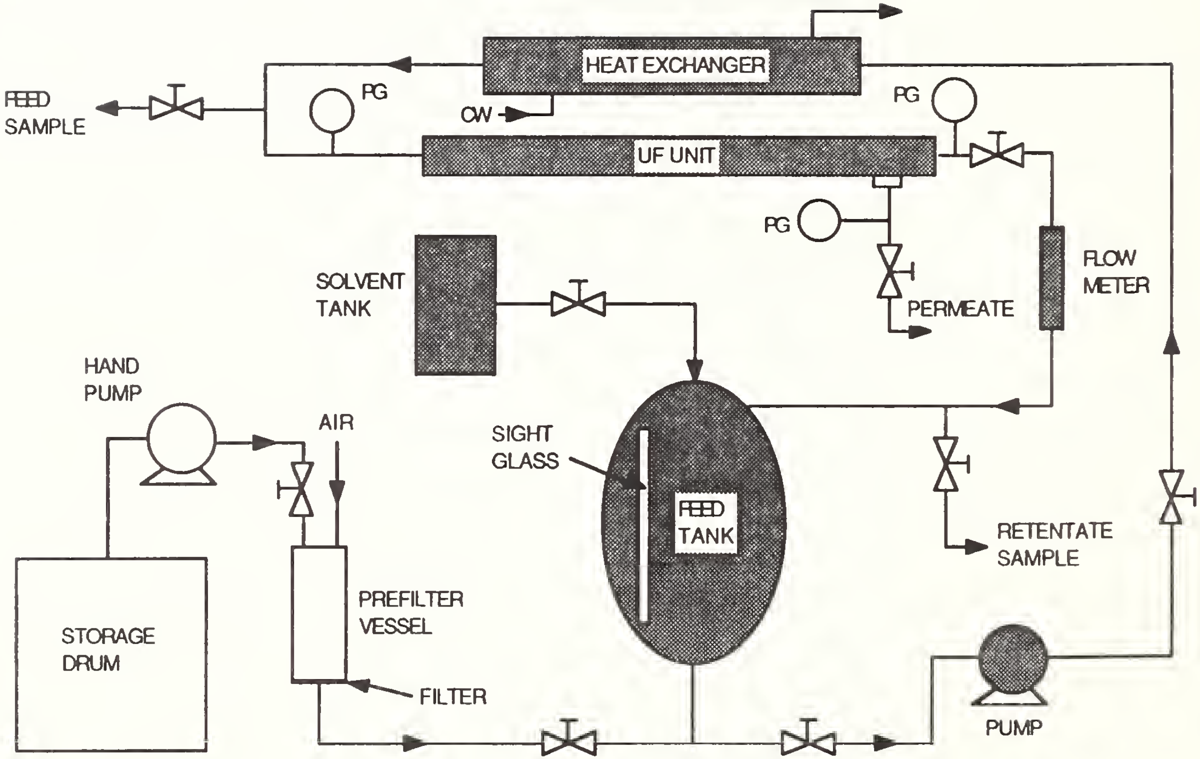

Figure 2. Ultrafiltration apparatus.

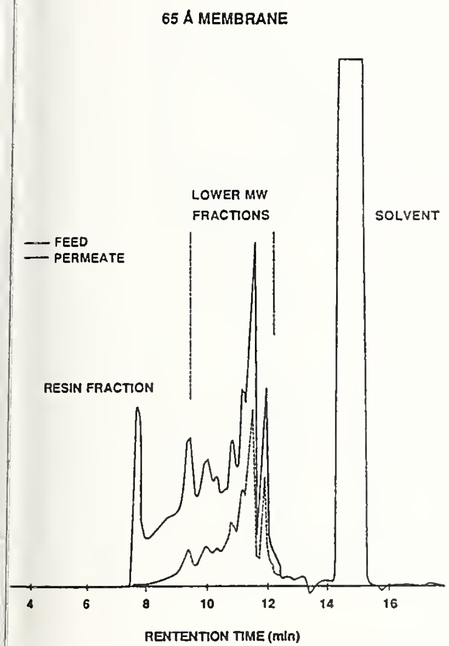

Figure 3. Size-exclusion chromatographic (SEC) results.

Electrostatic Separations

The first objective of this study was to demonstrate satisfactory performance (i.e., comparable to the data in References 11-13) for the Texas A&M electrostatic coalescer (Figure 4) in the resolution of well-defined oil-water-surfactant stabilized emulsions. The evaluation was directed toward obtaining information regarding the feasibility of resolving a stable emulsion, the throughputs for different voltages and frequencies, the minimum electrical potential under which coalescence is observed, and the maximum electrical potential before induced arcing or dielectric breakdown occurs.

In the configuration used, AC fields with voltages up to 20 kV (rms), frequencies between 45 and 3,000 Hz, and current draws of up to 250 mA were achieved. Provisions for pulsing direct current (DC) and unidirectional or filtered DC were also provided but were not evaluated. The system used two Elgar model 3001 power sources, one equipped with a variable frequency oscillator that provided the variable frequency signal at up to 115 V AC. The high voltage was obtained by the use of a Nothelfer Winding Laboratories (NWL) single phase step-up transformer connected in series with the Elgar AC power sources. The transformer was rated to increase the voltage from 115 to 20,000 V AC (rms) at a power of 5 kVA.

The potential and frequency of the sinusoidal waveform at the electrode was continuously monitored using Channel 1 on a Tektronix 2246A 100 MHz oscilloscope. The current was transported through the solution from the bottom plate to ground as a result of the imposed potential gradient. It was monitored through a 1 Ω resistor on Channel 2 of the oscilloscope. System details are given in Reference 12.

Various coalescers with cylindrical and rectangular geometries have been developed. Figure 5 is a schematic of a cylindrical coalescer vessel (12.9 l capacity, 0.178 m diameter [7.0 in.], and 0.521 m length [20.5 in.]) used in this phase of research. The principal components are: coalescer vessel, main body, emulsion distributor pipe, electrode, ground plate, and tubing connectors.

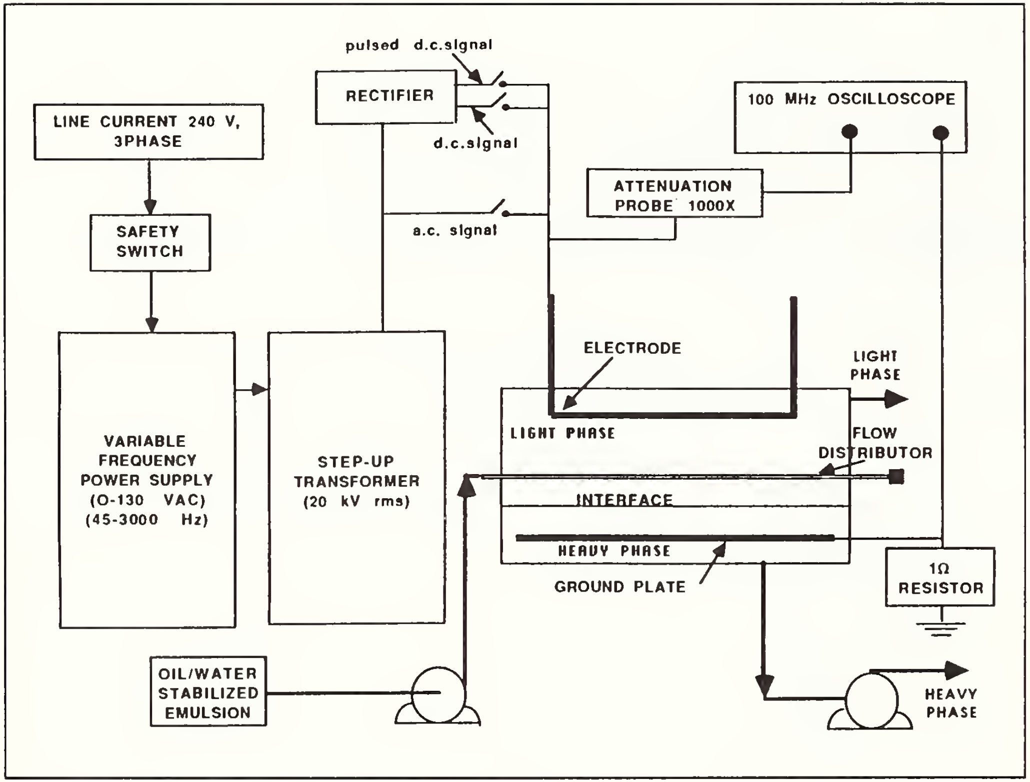

Figure 4. Schematic of the Texas A&M electrostatic coalescence system.

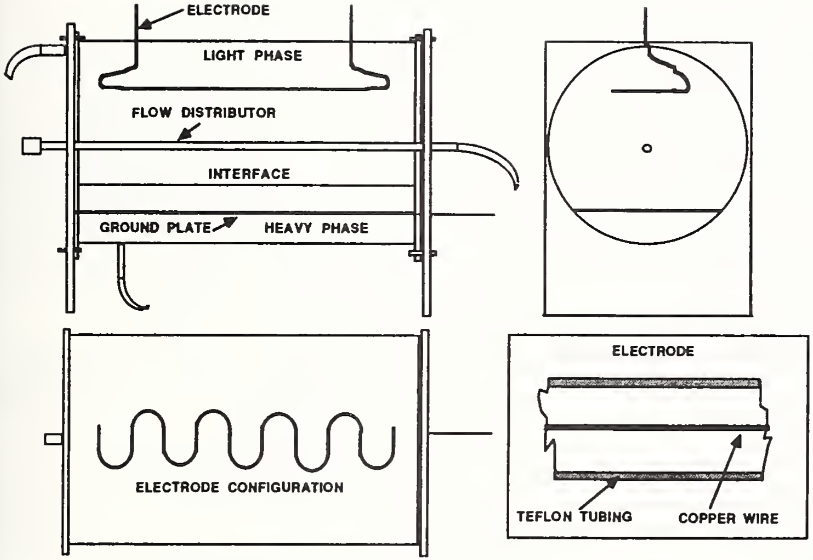

Figure 5. Schematic of cylindrical coalescer design.

The coalescer main body, as well as the emulsion distributor pipe, are made of Plexiglas® for flow visualization purposes. The distributor pipe is located at the axis of the coalescer and horizontally discharges the flow through a set of orifices. The orifices were designed to produce a uniform flow pattern along the length of the distributor pipe (14).

The emulsion that was pumped through the distributor pipe was continuously separated into its oil and water components to obtain a coalescence or throughput rate. After separation, the oil phase migrated to the upper region while the water phase went to the lower portion of the coalescer. Overflows were discharged separately at the top and the bottom of the vessel through control valves and associated tubing. Figure 6 shows a conceptual illustration of the emulsion input and the oil (light phase) and water (heavy phase) flows.

Isopar M was chosen as the continuous phase and water as the conductive component. Reproducible initial conditions, in the absence of phase disengagement (for at least 48 hours), were obtained by controlling the mixing parameters and using surfactants.

The conductivity of the light oil was measured by using the steady voltage conductivity cell method (17). The cell constant was determined by using a Gen-Rad 1688 AC conductance bridge. Specific conductivity was calculated from readings of electric current that circulates through the cell for an applied DC voltage. A 610C Keithley electrometer was used to measure electric current.

With the electric field totally established, emulsion was pumped at a gradually increasing volumetric flow rate, until a thin band of dispersion was observed at the interface. The coalescence rate obtained under these conditons was considered the maximum volumetric flow rate that the vessel could accept for total emulsion resolution. This was measured by timing the volume of oil and water collected in two graduated bottles.

Applied electric fields between 5 and 15 kV (rms) in combination with frequencies between 60 and 2,000 Hz were able to effectively separate the test emulsion under study. A graphical representation of the average coalescence rate (Figure 7) shows that the separation rate is more dependent on voltage than on frequency. Although the capabilities of separation increased only 1.7 times when the frequency alone changed, increments of up to 19 times were observed for variations in voltage. Moreover, below 10 kV (rms) no differences in rate of separation were apparent for 60 and 500 Hz.

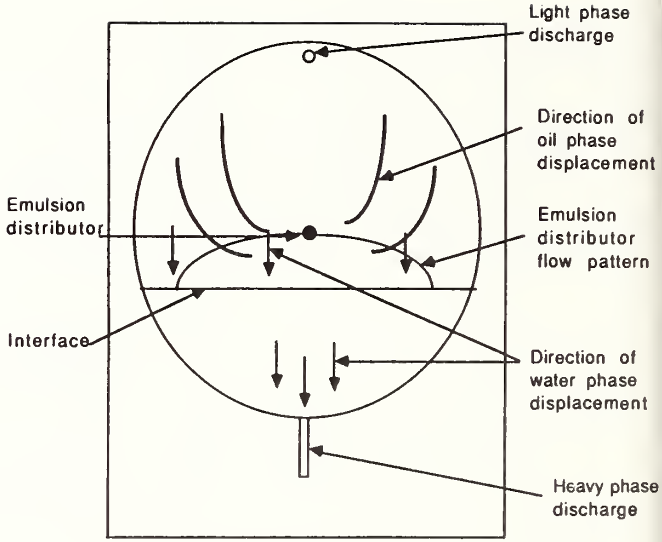

Figure 6. Conceptual illustration of emulsion input and separation of light and heavy phases.

Resin Purification Demonstration

The concept of crude oil desalting was investigated in a novel application for removal of salts and aqueous fraction solubles from guayule resins. Texas A&M resins from the flash evaporator takeoff (shown in Chapter 11, Figure 2) served as a realistic test fluid. Distilled water (10 micromho/cm electrical conductivity) was blended at a proportion of 10 percent by volume in the resin fraction by using a Tekmar Super Dispax Model SD45SN homogenizer. Coalescence was enhanced by adding Exxon surfactant Breaxit 7151 in the amount of 0.55 percent by weight of resin. A new 1.5 l glass coalescer vessel with vertical cylindrical geometry was fabricated with a redesigned proprietary delivery and takeoff system. A spiral electrode design,

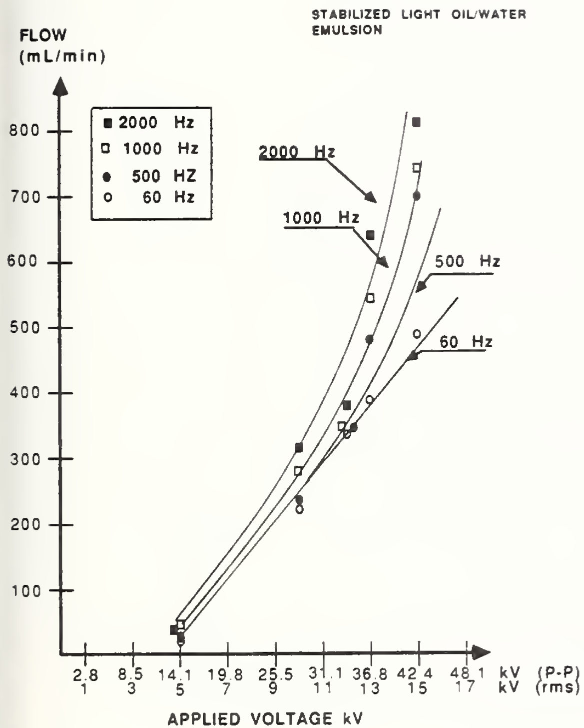

Figure 7. Coalescer separation for different voltages and frequencies.

which allows a more uniform electrical field distribution over the entire cross-sectional area of the vessel, was employed at 15, 20, 30, and 40 kV AC (peak to peak [p-p]) at frequencies of 150, 300, 600, 1,000, and 2,000 Hz.

The surfactant-treated resin/water emulsion was readily resolved under the imposed fields given above. Under only gravitational effects, very slight separation occurred after about 48 hours. Salt and/or aqueous fraction solubles transfer from the resin to the aqueous phase was verified by a dramatic increase in electrical conductivity and strong color changes in the aqueous phase. Table 3 shows aqueous phase conductivities up to 450 micromho/cm. This represents an increase of up to 45 times the conductivity of the distilled water (10 micromho/cm) used to prepare the emulsion.

The electrical conductivity of the resin fraction did not show a reduction after coalescence testing (Table 3). This may be due to the behavior of weak electrolytes in resin mixtures, which allows only partial ionization to occur. It might also be due to the presence of the surfactant, which is insoluble in the aqueous phase. The exact reason is not known at this time.

Figure 8 presents coalescence rate data for a range of voltages and frequencies. A maximum rate of 280 ml/min was measured at 40 kV (p-p) and a 1 kHz frequency. A value at 40 kV (p-p) and 2 kHz could not be measured due to experimental difficulties. Voltage appears to have a stronger effect on phase separation than frequency. The data also suggest that the influence of frequency is more important at low (between 150 and 600 Hz) than at high frequencies (between 1 and 2 kHz).

Coalescence of guayule resin emulsions on a simple volumetric basis appears to be easier than coalescence of model Isopar M emulsions examined previously. The maximum coalescence rate achieved in the 12.9 l coalescer with Isopar M was 810 ml/min at 2 kHz and 15 kV rms (i.e., 42.4 kV [p-p]).

Table 3. Electrical conductivity changes for coalescence of Texas A&M resins.

Component	Electrical conductivity (micromho/cm)[a]			
	Trial A	Trial B	Trial C	Trial D
Water fraction (before coalescence)	10	10	10	10
Water fraction (after coalescence)	300	380	400	450
Resin fraction (before and after coalescence)	4.5 x E-02	4.5 x E-02	4.8 x E-02	4.8 x E-02

[a] Steady voltage conductivity method for resin fraction; high frequencyAC for aqueous phase.

With guayule resins a rate of 280 ml/min was measured at 40 kV (p-p) and 1 kHz. The 8.6:1 ratio for the vessel used with Isopar M yields the maximum throughput ratio of only 2.9:1.

Bagasse Utilization Research

Solvent-extracted guayule bagasse affords the potential for use as biomass fuel in the production of process steam, feedstock for gasification and conversion to liquid hydrocarbons, a cellulosic base for a simple extruded fireplace log, and a base for hardboard overlays. These applications are not unique to guayule and as such must compete with other types of waste cellulose.

Thermal disposition of a few thousand tons/day (t/d) of guayule bagasse generated in commercial-scale processing facilities must be considered during periods of unfavorable economics for fireplace logs, fiberboard overlays, or other products. (In commercial facilities 25,000 t/y of product rubber produces about 364,500 t/y of bagasse [3].) A detailed study is required to determine the principal pollutants generated in various combustion processes and the means for their reduction. Dispersal of the pollutants into the atmosphere and the effects of

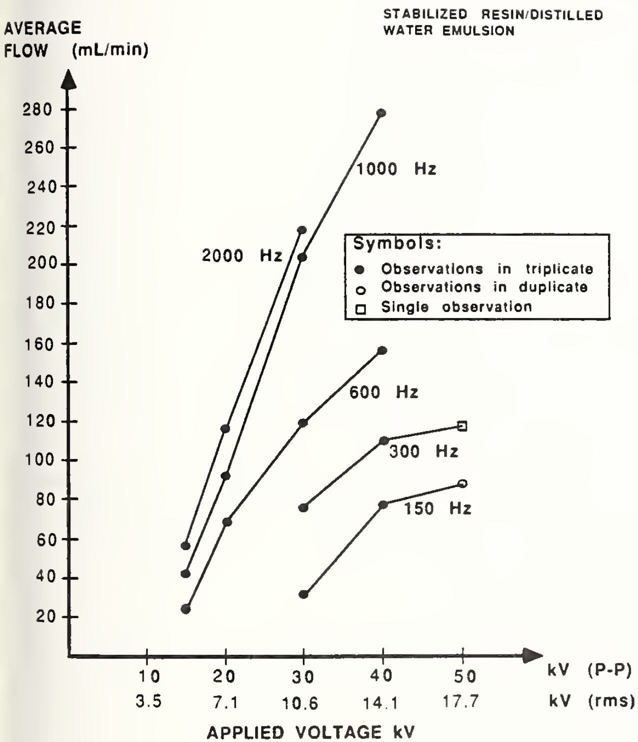

Figure 8. Coalescer separation of guayule resin for different voltages and frequencies.

consequent pollution upon the natural, industrial, and economic environments also requires careful evaluation.

The present study was limited in scope to a determination of toxic species resulting from combustion of a paraffin-based extruded guayule fireplace log. The following information was determined:

- Particulate smoke mass and heavy metals identification.
- Particle-size distribution.
- Identification of gases and condensable liquid and solid smoke particulates.

The underlying test goal was to determine whether any unusually toxic species were generated. Production of toxic species could eliminate the fireplace log as a viable coproduct or make it necessary to require specialized combustors or antipollution equipment for mass burning of guayule in commercial-scale processing plants.

The methods and procedures used in smoke mass analysis, particle-size distribution determinations, and chemical and heavy metals analyses are detailed in References 18-20. This chapter therefore describes only the principal highlights.

The smoke mass was determined by the use of an Arapahoe smoke chamber, as per ASTM D 4100 "Standard Method for Gravimetric Determination of Smoke Particulates from Combustion of Plastic Materials." This method differs from the widely accepted test method, which requires optical measurement of smoke produced and uses a National Institute of Standards and Technology (formerly the National Bureau of Standards) smoke chamber.

Particle-size distribution of the smoke was determined with a Sierra model 216 Cascade impactor, marketed by Anderson Samplers Incor-

porated. The stainless steel impactor has six radially slotted stages and a final filter. A Gillian model HFS 513 was the sampling pump.

Gas chromatography and mass spectrometry were chosen to evaluate compounds present in the smoke matter trapped in the filters. Samples were taken under varied combustion conditions including reduced air flaming combustion and reduced air smoldering combustion. Tetrahydrofuran (THF) was used as a solvent to remove particles from the filter surface. The resulting liquid was concentrated and filtered (0.47 μm) before being analyzed.

Table 4 shows the particle size distribution for three different combustion modes. For each combustion mode, cut-off points were calculated according to the procedures recommended by

Table 4. Particle-size distributions for different combustion modes of guayule fireplace logs.

Flaming[a]			Flaming/smoldering[b]			Smoldering[c]		
$D_{p,5}$ μm	Mass mg	Cum., % $<D_{p,50}$	$D_{p,50}$ μm	Mass mg	Cum., % $<D_{p,50}$	$D_{p,50}$ μm	Mass mg	Cum., % $<D_{p,50}$
1.2	0.48	77.9	11.2	0.61	76.4	1.1	0.83	40.7
2.2	0.28	86.6	2.1	0.30	86.1	2.0	0.35	77.6
3.5	0.17	91.8	3.3	0.23	90.2	3.1	0.09	93.2
6.0	0.14	94.8	5.5	0.15	93.8	5.2	0.04	97.3
14.0	0.03	97.3	13.7	0.17	96.1	13.0	0.003	99.2
23.5	0.03	99.5	23.0	0.07	98.3	21.0	0.01	99.3
0-1.2[d]	4.8	—	0-1.2[d]	6.54	—	0-1.2[d]	0.92	—

[a] Sampling rate = 5 l/min; sampling period = 8.33 min.
[b] Sampling rate = 5 l/min; sampling period = 8.33 min.
[c] Sampling rate = 5 l/min; sampling period = 3 min.
[d] Backup filter.

the manufacturer. Cut-off points are designated as $D_{p,50}$, which is the size of a spherical particle of density 1 gm/cm^3 that has the same terminal settling velocity as the sampled particle. The dimensions of $D_{p,50}$ are in micrometers.

Table 5 presents the results of qualitative emission spectrographic analysis. The positively identified elements were separated into the categories of major, minor, and trace. No major components were identified. Minor components are probably in the 100 to 1,000 ppm range, while trace components are most probably less than 100 ppm (A. Krawetz, Phoenix Chemical Laboaratory, Chicago, 1988, personal communication).

Except for chromium in the hexavalent state, no unusually toxic constituents were found in the smoke particulates, as gases or as condensable liquids, from flaming or smoldering combustion of guayule fireplace logs. The data simulates the gross results that would be obtained during actual burning in a fireplace. The results also indicate that modern combustion technology, which would be employed for mass burning of spent guayule bagasse in a commercial-sized plant, would not lead to unusually toxic species.

Guayule Bagasse as a Hardboard Overlay Material

Reports that the levels of accessible sugars in guayule bagasse are low in comparison to other sources of lignocellulose and the difficulty in hydrolysis of guayule's hemicelluloses prompted a cursory investigation of guayule bagasse as an overlay material in the production of exterior hardboard (21, 22). Exterior hardboard is manufactured by several companies, including Masonite, Weyerhauser, Georgia Pacific, and Abitibbi. Exterior hardboard is used in the construction industry for exterior siding on residential homes, condominiums, and in some cases, commercial buildings. It is reconstituted wood, formed under high pressure and heat into a variety of shapes and/or patterns depending upon the press-plate design and the manufacturing conditions. It is of major economic significance in the construction industry. Mr. Edwin Grissom of the Masonite Corporation, Laurel, Mississippi, prepared several laboratory samples

Table 5. Emission spectrographic analysis of guayule fireplace log samples.

	Elements detected		
Sample	Minor	Trace	Comments
Blank		aluminum, calcium, copper, magnesium, silicon, sodium	Lines used as reference
Flaming guayule sample A[a]	calcium, iron, potassium, silicon	aluminum, boron, copper, magnesium, lead, silver, strontium	All lines are stronger than blank
Smoldering guayule sample A[a]		aluminum, boron, calcium, copper, iron, magnesium, silicon, sodium	All lines are comparable to blank but no boron in blank
Flaming guayule sample B[b]	boron, iron, silicon, sodium	aluminum, calcium, chromium copper, magnesium, manganese, zinc	All lines are stronger than blank but no boron, chromium, manganese, or zinc in blank
Smoldering guayule sample B[b]	silicon	aluminum, boron, calcium, copper, iron, magnesium, sodium	All lines are stronger than blank but no boron in blank
Cotton gin trash sample[b]		aluminum, boron, calcium, chromium, copper, iron, magnesium, silicon, sodium	Aluminum, iron, and magnesium are comparable to blank but the remaining lines are stronger than the blank

[a] Sample collected on Whatman 41 filters.
[b] Sample collected on Whatman 42 filters.

of smooth, exterior 3/8-in.-thick hardboard containing guayule bagasse as the overlay material. The overlay material is responsible for the exterior features of the formed hardboard (i.e., its smooth versus rough texture). Scientists at the University of Southern Mississippi have compared the guayule overlay hardboards to "normal" production hardboard of the smooth-surface type. Scanning electron microscopy was utilized to study surface morphology and water and surfactant resistances were also determined.

The surfaces of the "guayule overlay" hardboards were smooth, glossy, and of high integrity. They were darker in color than the "normal" production hardboard, and their water and surfactant resistances were superior. This cursory, yet promising, study indicates the need for more extensive investigation of guayule bagasse as an overlay material for exterior hardboard siding.

RECENT DEVELOPMENTS IN POLYMER SCIENCE

Chlorination of low-molecular-weight guayule rubber produces coating-grade chlorinated rubber. Carbon Nuclear Magnetic Resonance Spectroscopy (^{13}C NMR) and elemental analysis confirm that guayule chlorinated rubber's chemical structure and composition are similar to that of commercial-grade chlorinated rubber. The use of Azo-bis-isobutyronitrile (AIBN) as a chlorination catalyst significantly reduces molecular weight and results in the formation of lower viscosity grades of chlorinated rubber.

Chlorination of Low-Molecular-Weight Rubber

Investigators at the University of Southern Mississippi have evaluated the utility of the low-molecular-weight guayule rubber fraction as a raw material for the production of chlorinated rubber. As a result, the synthesis and characterization of chlorinated low-molecular-weight guayule rubber was reported for the first time (23). In a typical experiment, the chlorination of

natural rubber involves two steps: (1) mastication of high-molecular-weight rubber to a reduced molecular weight followed by (2) chlorination. Mastication is required because viscous solutions of natural rubber create manufacturing difficulties, including gel formation and heat buildup. The mastication procedure reduces the solution's viscosity to a manageable level.

Therefore, the low molecular weight of naturally occurring guayule rubber is an advantage because mastication is not required prior to chlorination. Low-molecular-weight guayule rubber was isolated by treating guayule resin with 90 percent ethanolic solution. This resulted in the separation of low-molecular-weight rubber as a solid. The raw rubber was purified by dissolution in a 5 percent carbon tetrachloride solution and reprecipitation with the addition of a 90 percent ethanolic solution. The purification process was monitored by ^1H NMR (Proton Nuclear Magnetic Resonance Spectroscopy) and ^{13}C NMR.

The chlorination of guayule rubber was affected in a 5 percent carbon tetrachloride (CCl_4) solution in a three-neck flask fitted with a water condenser, gas dispersion tube, and adapter. The gas dispersion tube was connected via Teflon® tubes to a chlorine cylinder through two gas traps. The exit port of the condenser was connected via Teflon tubing to ice-cooled traps containing 2 N sodium hydroxide solution (NaOH). The reaction flask was immersed in an oil bath for temperature control. Nitrogen gas (N_2) was purged through the system for 10 minutes to ensure complete removal of oxygen. A blanket of N_2 was maintained over the reaction throughout the chlorination process. The solution was allowed to reflux at 79° C with constant stirring via a magnetic stirrer. A slight excess of chlorine gas (10 percent mol excess) was allowed to bubble through the solution and the liberated hydrogen chloride (HCl) was trapped in the sodium hydroxide solution. The chlorinated rubber product was isolated as a white precipitate with the addition of 90 percent ethanol solution.

Fourier Transform Infrared Spectrocopy (FTIR) spectra were recorded on a Mattson, Polaris, spectrometer. Films were cast by the evaporation of a toluene solution of chlorinated rubber. Drying the films in a vacuum oven ensured the removal of all solvent.

^1H NMR and ^{13}C NMR were obtained from a 300 MHz Brucker fourier transform spectrometer. The solutions (20 percent weight/weight [w/w]) were prepared by dissolving the chlorinated rubber in $CDCl_3$ and C_6D_6 for the ^{13}C NMR spectra analysis with tetramethylsilane as an internal standard.

Gel permeation chromatograms were generated from a Waters Associates, Inc., GPC equipped with a refractive index detector. The operating conditions included: mobile phase, tetrahydrofuran (THF) at a flow rate of 1 ml/min with columns 106, 104, 500, and 100 Å. Sample concentrations were prepared at 0.2 percent (w/w); a 100 µl aliquot was used for molecular-weight analysis. Standard polystyrene samples from Polymer Laboratories, Inc., were used to create a calibration curve.

Thermal analyses were performed on a Dupont Model 9900 thermal analyzer under nitrogen atmosphere. A heating rate of 10° C/min was employed for T_g determinations.

Duplicated elemental analysis of chlorinated rubber samples was carried out by the MHW Laboratory, Phoenix, Arizona.

Results and Discussion

The synthesis of chlorinated rubber from low-molecular-weight guayule rubber has occurred and its structure has been verified. Other investigators (24) have performed similar studies on the formation of chlorinated rubber from *Hevea* rubber. An empirical formula of $C_5H_8Cl_{3.5}$ determined for the product indicates that chlorination involved more than one isoprene unit [CH_2=CH-CH(CH_3)=CH_2]. These products were soluble in organic solvents, lending additional support to the thesis that cyclization rather than cross-linking is the predominant reaction (25). The chlorine content of the products was 65 percent. This is consistent with a combination of substitution and addition reactions followed by cyclization (24). The concept of cyclized units along the polymer backbone is supported by FTIR and ^{13}C NMR analysis (26).

The physical and mechanical properties of chlorinated rubber are often determined by its chlorine content and molecular weight. For instance, lower-molecular-weight chlorinated rubber is used for printing inks and higher-molecular-weight chlorinated rubber is necessary for coating applications. We have found that the chlorination of low-molecular-weight guayule rubber principally yields coating-grade chlorinated rubber.

Guayule rubber is contaminated with wax and other hydrocarbons and must be purified before chlorination. In this study the purification process was monitored by proton NMR spectroscopy (Figure 9). The peak assignments representing satisfactory purification are as follows:

1.67 ppm (*Cis* double bond methyl protons)
2.00 ppm (methylene protons)
5.12 ppm (vinyl proton)

The small peak at 1.25 ppm represents an impurity(ies) not removed during the extraction process. Further purification from an ethanolic solution reduces the impurity(ies) to an insignificant level.

The various experimental chlorinating conditions are summarized in Table 6. The radical initiator, Azo-bis-isobutyronitrile (AIBN), was used in catalytic amounts to determine its

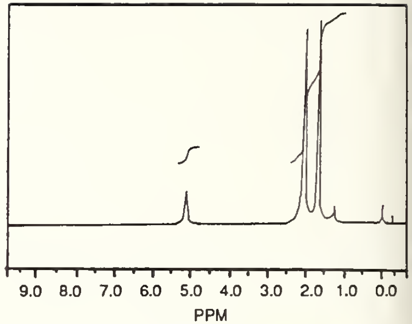

Figure 9. ^1H NMR of purified guayule natural rubber.

Table 6. Experimental conditions used in the synthesis of chlorinated rubber.

Batch no.	Amount of guayule rubber used (g)	Amount of chlorinated rubber isolated (g)	Cl (%)
1[a]	3.20	6.00	59.81
2[b]	5.00	11.80	60.00
3[c]	5.00	13.14	63.67

[a] Solvent; CCl_4, 200 ml.
[b] Solvent; CCl_4, 100 ml.
[c] Solvent; CCl_4, 100 ml and 37.5 mg of AIBN.

efficacy, if any, in effecting changes in the chlorine content of the products. Recent studies of rubber chlorination have reported that AIBN increases the rate and amount of chlorination. In our case, the use of either purified and/or partially purified guayule rubber provided essentially identical amounts of chlorination, that is, 60 percent. The lower yield (6.00 g) obtained in the first instance is attributed to the presence of impurities in the starting materials, while purified guayule rubber (Figure 10) gave a significant increase in product yield. The use of AIBN increased the chlorine content to a value approaching theoretical for a fully chlorinated rubber (64.7 percent). Azo-bis-isobutyronitrile was instrumental in increasing the degree of chlorination, although its mechanistic role is not fully understood.

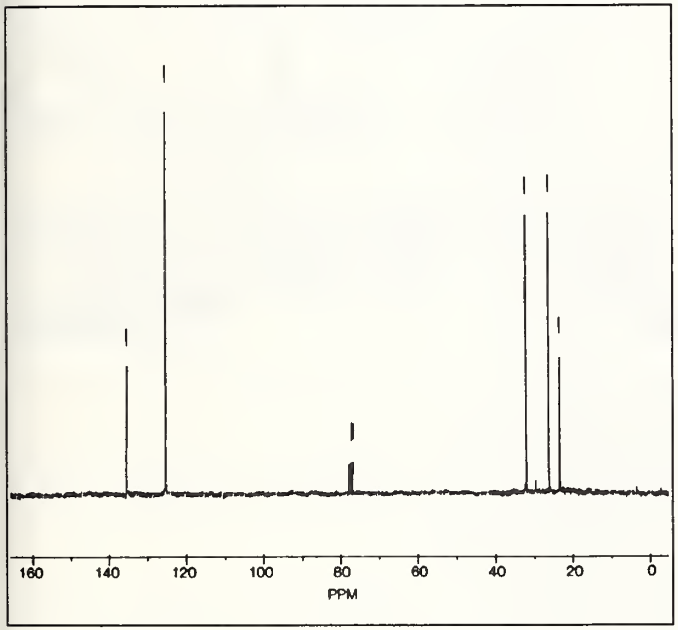

Figure 10. ^{13}C NMR spectrum of guayule rubber in $CDCl_3$.

The FTIR spectrum of Figure 11, which compares a commercial grade of rubber (Alloprene CR-20 from Imperial Chemical Industries [ICI]) with guayule chlorinated rubber, confirms their essentially identical composition. Absorption bands characteristic of chlorinated rubber appear near 780/cm and 736/cm and represent the secondary C-Cl and the CH_2 rocking frequency (26), respectively. The absorbance bands at 2,939/cm, 1,440/cm, and 1,260/cm are due to the C-H stretching and bending absorptions, respectively. The weak absorbance near 1,630/cm indicates residual unsaturation.

The major ^{13}C NMR chemical shifts of guayule chlorinated rubber are shown in Table 7 (26); the spectrum is displayed in Figure 12. To identify the unsaturated carbons, spectra were taken in $CDCl_3$ solvent, and peak assignments were based on spectra obtained for chlorinated hydrocarbons of known structure (27-28) (Commercial-grade CR-20 and CR-5 were obtained from Polyvinyl Chemical Company [ICI Resins US], Wilmington, Maryland). The values for guayule chlorinated rubber chemical shifts are in excellent agreement with the litera-

Table 7. Characteristics of ^{13}C NMR spectra of guayule chlorinated rubber and commercial-grade chlorinated rubber.

Chemical shifts (ppm)		
Guayule rubber	Literature	Assignment
21.5, 28.7, 34.7, 37.6	21.5, 28, 34.7, 37.4, 21.5, 38, 37.4	CH_2, CH_3
45.4, 48.01	45.4, 48	$-CH_2Cl$
62.25, 64.37	62—64	= CHCl
75.1, 77.18	74—77	= CCl

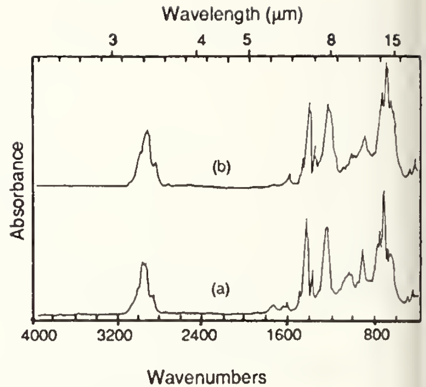

Figure 11. FTIR spectrum of chlorinated rubber: a) guayule chlorinated rubber, and b) commerical-grade chlorinated rubber.

ture values reported for commercial-grade chlorinated rubber (26). Makani and coworkers (26) concluded that the broad peaks observed in ^{13}C NMR spectra are a result of a variety of structures present in chlorinated rubber, which makes its chemistry complicated. The peaks at 74 ppm and 77 ppm are due to quaternary carbons linked to a single chlorine atom. The CHCl group appears at 63 and 64 ppm.

Gel permeation chromatograms of the various guayule chlorinated rubber products are shown in Figures 13 and 14, with a chromatogram of commercial grade Alloprene CR-20 (CR-20) chlorinated rubber included for comparative purposes. The guayule chlorinated rubber and commercial-grade CR-20 are of the same molecular-weight range and molecular-weight distributions (Figure 13), except that the commercial CR-20 exhibited a low-molecular-weight shoulder. Since CR-20 is used primarily in the coating industry, especially in traffic paints and marine coatings, these data suggest a similar use for guayule chlorinated rubber. Guayule chlorinated rubber seems particularly suited for this purpose because it forms continuous, transparent films from toluene solutions (20 percent solution). In contrast, guayule chlorinated rubber, obtained in the presence of AIBN, has a lower molecular weight than guayule chlorin-

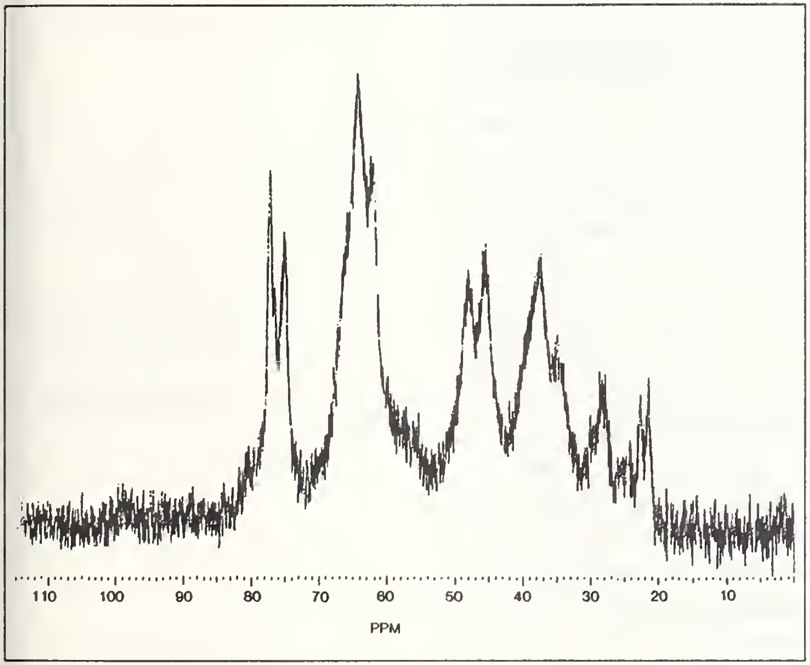

Figure 12. [13]C NMR spectrum of guayule chlorinated rubber in C_6D_6.

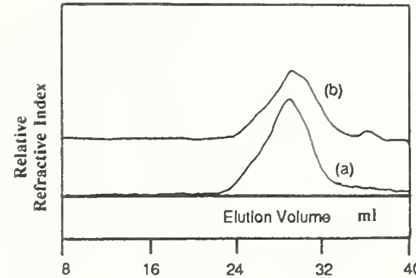

Figure 13. Gel permeation chromatograms of chlorinated rubber: a) guayule chlorinated rubber, Cl ~ 60 percent, and b) commercial-grade chlorinated rubber, Cl ~ 64-65 percent.

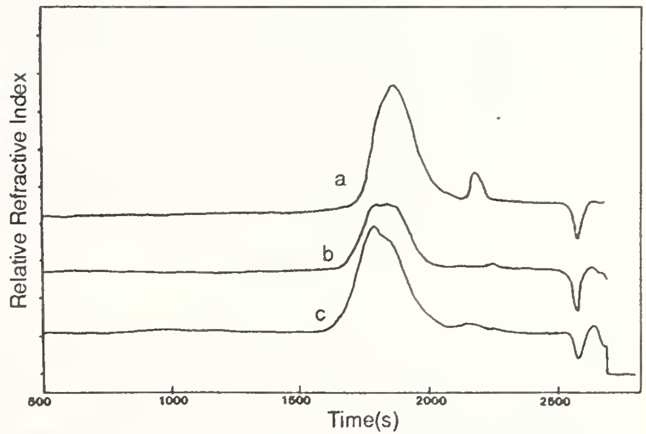

Figure 14. Gel permeation chromatograms of chlorinated rubber: a) guayule chlorinated rubber prepared with AIBN, excess chlorine, b) guayule chlorinated rubber prepared with AIBN (Cl; 63.7 percent), and c) commercial-grade CR-5 (Cl; 64-65 percent).

ated rubber prepared without AIBN. Its molecular weight corresponds to that of commercial-grade Alloprene CR-5 (CR-5) (Figure 14), which is used primarily in the printing ink industry. It is clear therefore that the use of AIBN results in a slight increase in the chlorine content with a concomitant and significant reduction in molecular weight. Films prepared from CR-5- and AIBN-derived guayule chlorinated rubber were found to be brittle and difficult to remove from the steel panels. These results indicate that guayule low-molecular-weight natural rubber can be successfully chlorinated in a fashion similar, if not identical, to that of *Hevea* natural rubber or synthetic poly *cis*-isoprene.

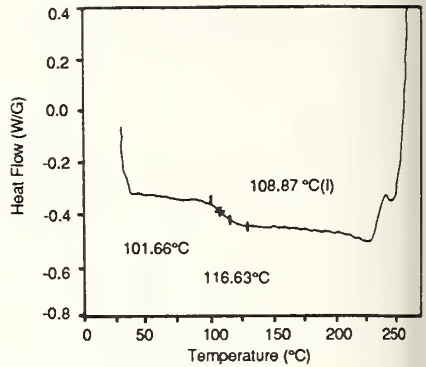

Figure 15. DSC of guayule chlorinated rubber.

The Differential Scanning Calorimetry (DSC) spectrum of guayule chlorinated rubber with 60 percent chlorine content (Table 6, batch 1) is shown in Figure 15. The glass transition temperature (T_g; the temperature at which a polymer turns from a glassy to an amorphous state) of guayule chlorinated rubber is approximately 108° C, while the T_gs are 126° C and 128° C for CR-5 and CR-20, respectively. The lower T_g values for guayule chlorinated rubber may be due to traces of waxy materials that act as plasticizers and reduce the glass-transition temperature.

In summary, low-molecular-weight guayule rubber can be easily chlorinated and gives coating-grade chlorinated rubber. ^{13}C NMR and elemental analysis of the chlorination products have shown its chemical structure and composition to be similar to that of commercial-grade chlorinated rubber. The use of AIBN during chlorination significantly reduces molecular weight, which yields lower viscosity grades of chlorinated rubbers.

Guayule Resin as an Adhesion Modifier

University of Southern Mississippi scientists have shown the utility of guayule resin as an adhesion modifier for coatings derived from epoxy resins. Unmodified epoxy resin-based coatings characteristically have excellent physical, mechanical, water, and corrosion resistance properties combined with superior adhesion. However, there are applications where the physical, mechanical, and environment resistant properties are desirable yet a selective decrease

in adhesion is needed, for example, in the case of strippable coatings. Strippable coatings are used for a variety of applications that require temporary protection and ease of removal; for example, during shipping and storage. In the latter case, aircraft and other military vehicles placed in storage are excellent examples. Strippable coating(s), which have excellent protective properties and can be easily removed without use of organic solvents or solutions of acids or bases, are in high demand. Guayule resin can be used to reduce the adhesive character of epoxy resin-based coatings, which retain excellent physical, chemical, and environmental resistant qualities. The following is a description of the synthesis and evaluation of strippable coatings derived from an epoxy resin (Epon 828) and guayule resin.

Materials and Methods

Epon 828 (Shell Chemical Company, USA) is the diglycidyl ether of Bisphenol A, with an epoxide equivalent weight of 188. That is, every 188 g of the diglycidyl ether molecule contain, on the average, one epoxide group; the group that reacts with polyoxypropylene diamine to form the epoxy coating. It is necessary to know the epoxide equivalent weight in order to select the appropriate amount of diamine to affect a chain-growth or polymerization reaction.

The polyoxypropylene diamine (Jeffamine D-400, MW~400) used in this work was obtained from Texaco Chemical Company, USA. Guayule resin was provided by Bridgestone/Firestone, Akron, Ohio. Research-grade n-butanol and xylene were employed as solvents. Byk-Chemie USA supplied the flow control agent, Byk-341, which is necessary to form a film of high integrity with no film voids.

The strippable coating formulations were made by dissolving Epon 828 and guayule resin in a solvent mixture of n-butanol/xylene 50/50 (w/w), adding appropriate amounts of diamine and flow control agent, and then filtering through glass wool to remove traces of insoluble materials. The films were prepared on metal panels at a wet-film thickness of approximately 3 mils (1 "mil" = 1/1000 in.). Phosphatized steel ("Bonderite," Parker Chemical Company, USA),

cold-rolled steel, sand-blasted aluminum panels, and nontreated aluminum panels (Q-Panel Company, USA) were used for film castings. The coatings were cured at 150° C for three hours in a forced-air oven. Table 8 shows formulations of the coatings.

Table 8. Formulation of guayule modified epoxy coatings.

	Epon-Amine/Guayule resin (w/w)			
	100/0 (g)	95/5 (g)	90/10 (g)	80/20 (g)
Epon 828 (epoxy ether resin	10.00	10.00	10.00	10.00
Polyoxypropylene amine (Jeffamine D-400)	6.19	6.19	6.19	6.19
Guayule resin (Bridgestone/Firestone)	—	0.86	1.80	4.05
Solvent mixture (n-Butanol/xylene, 50/50)	6.89	7.30	7.71	8.67
Byk-341 (Byk Chemie)	0.18	0.18	0.18	0.18

Differential Scanning Calorimetry (Dupont Model 9900) was used to determine T_g. Glass transition temperatures are particularly important characteristics for organic coatings because they are indicators of the coating's ability to flow and wet the substrate at room temperature. Moreover, T_g is an indicator of the degree of flexibility the coating will possess in use; high T_g coatings will be brittle while lower T_g coatings will tend to be more flexible. Films were prepared for T_g determinations by casting on either cold-rolled steel or untreated aluminum panels. T_gs are reported as the midpoints of the endotherm curve of the DSC Scan (Figure 16). The T_gs are 32° C, 19.5° C, and 20.0° C for the epoxy:guayule resin compositions of 95/5 w/w, 90/10 w/w, and 80/20 w/w, respectively.

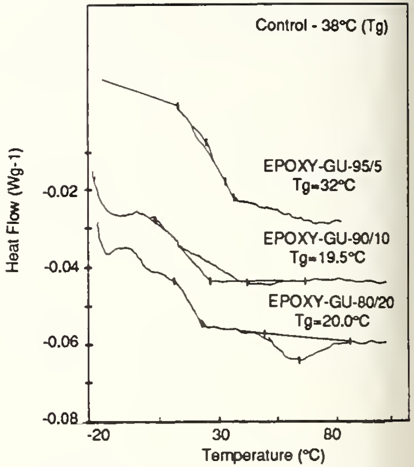

Figure 16. Effect of guayule resin on the T_g of epoxy coatings.

Selected physical properties were measured to determine the efficacy of the strippable coatings. An effective coating must be able to withstand impact and retain its physical integrity and adhesive bond to the substrate. Accordingly, the impact resistance of the coatings applied to metallic substrates was determined using a Gardner Impact Tester (Paul N. Gardner Company, USA). The film properties of guayule modified coating are reported in Table 9 as direct and reverse impact. The former represents the film's ability to sustain a falling weight directly upon its surface (i.e., a crushing effect) and the latter is a measure of the coating's ability to elongate rapidly when the substrate to which it is attached is deformed under the force of a falling object. The physical integrity or toughness of the coating is indicated by the determination of tensile strength and elongation measurements of the free or unadhered films. Tensile strength and elongation measurements were made with an Instron Testing Machine (Model A1020C, Instron Corporation, Canton, Massachusetts) and are recorded in Table 10. Test

Table 9. Film properties of guayule modified coating on treated panels.[a]

Epoxy-Amine/Guayule (w/w)	100/0	95/5	90/10	80/20
Thickness (mils)	1.5	1.5	1.5	1.5
Pencil hardness	H[b]	H[b]	H[b]	HB[b]
	H[c]	H[c]	HB[c]	B[c]
Resistance to boiling water (2 h)	No loss of adhesion or softening			
Direct impact resistance (J)	17[b]	17[b]	17[b]	15.9[b]
	5.67[c]	6.8[c]	5.67[c]	4.53[c]
Reverse impact resistance (J)	17[b]	15.9[b]	15.9[b]	—[b]
	6.8[c]	6.8[c]	5.67[c]	4.53[c]

[a] The solvent was xylene/Butanol 50/50 (w/w); the cure cycle was 3 h at 125° C; and the flow control agent was Byk-341 (Byk Chemie).
[b] Phosphatized steel substrate.
[c] Sand-blasted aluminum substrate.

specimens were cut into appropriate shapes using a die as per the American Society for Testing and Materials (ASTM) test procedure D-2370. Test samples of even thickness were selected by measuring the thickness with a caliper having a sensitivity of 0.0001 in. or 0.1 mil.

Results and Discussion

The reaction of amines, a family of nucleophilic reagents that includes polyoxypropylene diamine (Jeffamine D-400), with epoxy resins has been reviewed and studied in considerable detail (29, 30). As a result, it is known that the reactants, epoxy resins and primary amines, in

Table 10. Film properties of guayule-modified epoxy coatings on nontreated panels.[a]

Epoxy-amine/Guayule (w/w)	100/0	95/5	90/10	80/20
Thickness (mils)	1.5	1.5	1.5	1.5
Pencil hardness	H[b]	2B[b]	2B[b]	2B[b]
	H[b]	2B[c]	2B[c]	2B[c]
Resistance to boiling water	Film lost adhesion after 30 min			
Direct impact resistance (J)	17[b]	d	d	d
	6.8[c]	d	d	d
Reverse impact resistance (J)	17[b]	d	d	d
	6.8[c]	d	d	d
Tensile strength (Pa)	4.14×10^{7}[b]	4.0×10^{7}[b]	3.9×10^{7}[b]	
Elongation at break (%)	3.5[b]	3.6[b]	4.0[b]	

[a] Solvent was xylene/butanol 50/50 (w/w); the cure cycle was 3 h at 125° C; and the flow control agent was Byk-342 (Byk Chemie).
[b] Substrate was cold-rolled steel panels.
[c] Substrated was nontreated aluminum panels.
[d] The film released at these w/w levels.

the strippable coatings formulations form a three-dimensional network when cured. Figure 17 is an illustrative depiction of the reactants used in the formation of an epoxy strippable coating derived from Jeffamine D-400, Epon 828, and guayule resin. The variations in amounts of epoxy resin, amine, and guayule resin used in the strippable coating formulations are shown in Table 8. The amount of guayule resin was varied from 0 to 20 percent w/w in an effort to determine its effect on adhesion and other coating properties. Furthermore, to determine the effect of surface preparation on adhesion, phosphatized and surface-abraded aluminum panels were employed as substrates.

Table 9 is a compilation of test data, which include impact resistance, pencil hardness, and boiling-water resistance of unmodified and guayule-modified epoxy resins cast onto aluminum and steel substrates. The unmodified and guayule-modified epoxy coatings with up to 10 percent guayule resin showed similar performance characteristics when applied to treated steel and aluminum substrates. They exhibited excellent adhesion and good impact resistance and hardness. The films did not soften nor did they show adhesion losses even after immersion for two hours in boiling water. However, at higher levels of guayule resin use (20 percent w/w), film properties deteriorated.

In contrast, the films of the guayule/epoxy composition cast onto nontreated steel and aluminum panels were easily strippable (Table 10). However, the unmodified epoxy coatings were nonstrippable and exhibited superior adhesion. Water immersion of untreated steel and aluminum panels coated with epoxy/guayule resins resulted in loss of adhesion after 30 min in the boiling water test. The decline in impact resistance, pencil hardness, and boiling-water resistance are the result of poor adhesion to the substrate. For instance, these films were more prone to develop cracks on impact, to tear with softer pencil leads, and to lift from the surface under the influence of boiling water. Thus, the ease with which these coatings could be removed from the substrate has led us to formulate a class of novel epoxy strippable coatings whose illustrative compositions are reported in Table 8. It is significant that as little as 5 percent (w/w) incorporation of guayule resin engendered strippability to the epoxy coatings.

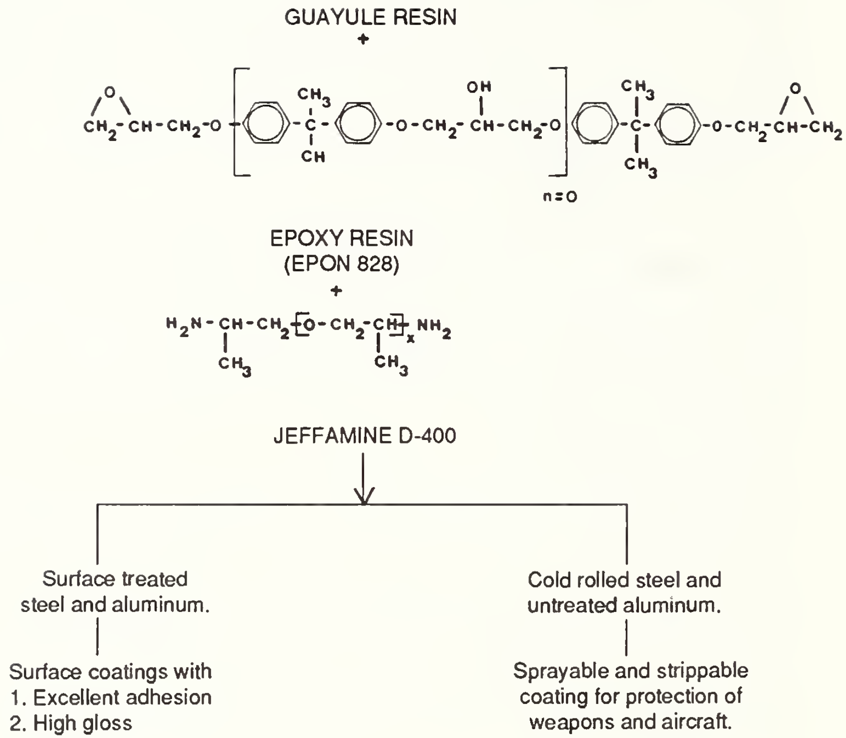

Figure 17. Formulation of epoxy coatings with guayule resin as a plasticizer.

The 10 percent guayule-modified epoxy films showed a slight decrease in tensile strength with a corresponding increase in percent elongation (Table 10). These results clearly illustrate two important findings.

First, in the case of guayule-resin-modified epoxy coatings, the surface treatment of panels dramatically improved the adhesion to the substrate. In contrast, the unmodified epoxy-resin coatings did not show noticeable changes in film performancewith either treated or untreated coated panels.

Second, when untreated panels, such as cold-rolled steel, are used guayule resin acts as an effective surface modifier for epoxy coatings, rendering them strippable.

This characteristic of guayule resin/epoxy resin formulated coatings is a unique application for the organic, soluble guayule resin extract and offers potential for commercialization. We believe that epoxy modification with guayule resin is a viable and economically feasible approach for the production of strippable epoxy resin-based coatings.

The effects of guayule resin on the T_g of films formed from modified epoxy resins is shown in Figure 16. The T_g of the control resin is approximately 38° C and the T_g of the 20 percent guayule-modified films is approximately 20° C. This significant drop in T_g for guayule-modified films is indicative of the plasticizing effect of guayule resin in epoxy coatings. Films with 20 percent guayule modification exhibit an endotherm at approximately 60° C, suggesting a melting transition for one of the resin components. Microscopy examinations were performed in an effort to study the surface morphology of the film matrix. Typically, the resin particles were small and nearly spherical in shape. However, the dispersed resinous phase assumed an irregular configuration and adhesion was reduced as the amount of guayule resin increased. Thus, higher levels of resin modification may lead to complete phase separation, which could limit the usefulness of guayule resin modification. Abramov and coworkers (31) noted that increasing mixing intensity has a profound effect on the shape of the dispersed particles. Higher mixing rates resulted in the dispersed particles assuming spherical configurations whereas at lower mixing rates larger particles with irregular configuration formed.

However, in the University of Southern Mississippi study, the resins were mixed under identical conditions and all films were cast from the same solvent mixture. Therefore, it was concluded that the observed irregular configuration resulted primarily from increasing incompatibility of guayule resin and epoxy resins as the concentration of guayule resin in epoxy resin increased. Films with 10 and 20 percent guayule resin have essentially identical T_gs, irrespective of the differing amounts of guayule resin in the formulation (Figure 16). Similar observations have been encountered during morphological studies of polymer alloys.

In summary, guayule resin can be employed as an adhesion modifier in amine-cured epoxy resins and its level of modification controls the degree of adhesion of the epoxy coating(s). Thus, judicious use of guayule resin in amine-cured epoxy formulations results in the production of strippable amine-epoxy coatings with exceptional properties (Tables 9 and 10).

Guayule Resin Extracts as Biologically Active Agents

Dr. Dale Bultman of the Naval Research Laboratories has performed extensive qualitative studies to confirm the presence of biologically active agents in the guayule resin fraction. As a result of his studies, scientists at the University of Southern Mississippi have undertaken the separation and isolation of guayule-resin components and the identification of the biologically active fraction(s). They have utilized thin-layer chromatography and column chromatography to isolate and separate thirty-two fractions. Fractions 23 through 32 were combined and possessed significant activity in biological assays against two wood-rotting fungii, *Phelinus gilvus* and *Lendius lepidus*. As yet they have not identified the specific structure of the biologically active agent(s), but their work is continuing.

CONCLUSIONS

Recent progress made in the separation and purification of the guayule processing components and in the development of high-dollar-valued coproducts affords the potential to offset growing and processing costs. Indeed, cultivating and processing cost reductions are necessary if the production of guayule rubber is to be competitive with that of *Hevea* rubber. Expanded research efforts are needed to eliminate the difficulties in coproduct separation and isolation, as are efforts emphasizing the utility of guayule components in polymer applications.

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Chapter 14

Guayule Economics

N. Gene Wright, Susan Fansler, and Ronald D. Lacewell

The history of guayule commercialization has been influenced more by economics than by any other single factor. When the economic climate is favorable, interest is stimulated and progress is made through research in developing guayule into a feasible industry. However, when either market prices or the availability of competitive elastomers reverse the economic situation, guayule projects are set aside. The following brief history of guayule in the United States and Mexico illustrates the importance of economics in the development of a guayule rubber industry.

A HISTORICAL SKETCH OF GUAYULE ECONOMICS

The first involvement of a U.S. company with guayule rubber production was in 1888. The New York Belting and Packing Company had 100,000 pounds of guayule shrub shipped from Mexico to New York City, where rubber was extracted by immersing the woody material in hot water. Unfortunately, the small amount of rubber obtained did not sufficiently offset the cost of transporting the material from Mexico, and so the project was dropped. By 1902, researchers funded by capital from Germany had developed a method to extract rubber from the guayule woody material using solvents. This process also proved too expensive for commercial use and was discontinued by 1905.

In 1904, a pebble mill process using mechanical means to extract the rubber was invented. The first commercial-size processing plant using this method was built in Torreon, Coahuila, Mexico, by the Continental-Mexican Rubber Company in 1905-06, and as many as 13 pebble mill plants were eventually built in that country. This method was used to process guayule in Mexico from 1906 to 1944. However, freight costs plus a Mexican export tax on guayule shrub material made the pebble mill method uneconomical for U.S. processing plants that used imported shrub material.

About 27.1 million pounds of guayule rubber was produced in Mexico between 1905 and 1945 (Table 1), and almost all of this rubber was shipped to the United States. In 1910, guayule provided 10 percent of the world's natural rubber needs and 50 percent of U.S. needs. It continued to be a minor source of commercial natural rubber until the end of World War II. Between 1906 and the Mexican Revolution of 1912, selling guayule shrub material became very profitable for landowners in Mexico; during this period ranchers in the guayule-producing area were receiving $100 per acre for the rights to harvest native stands of the shrub. Prices paid to harvesters for guayule shrub material delivered to the Mexican processing plants increased from $7.50 per ton in 1906 to $50 per ton by 1912. The wealthy Madero family, which controlled 2 million acres of native guayule, made millions during this period. Francisco Madero became president of Mexico from 1911 to 1913, financed partly from profits made harvesting and processing guayule for rubber.

Because harvesting native stands of guayule for rubber was so profitable, the Continental-Mexican Rubber Company warned in 1911 that domestication and cultivation were necessary or guayule would become extinct in Mexico. However, the danger was soon averted by the onset of the Mexican Revolution, during which many of the guayule processing plants were closed and the native stands thus saved from possible extinction. The significant economic impact of the revolution on Mexico's guayule industry can be seen in the guayule export data: in 1912 almost 13.9 million pounds of guayule rubber was shipped from Mexico to the United States, but in 1914 these shipments totaled less than 0.6 million pounds (Table 1).

Table 1. Mexican production of guayule rubber.

Year	Pounds	Year	Pounds
1905	750,000	1926	9,529,257
1906	3,637,500	1927	11,975,701
1907	8,610,000	1928	6,210,200
1908	10,863,750	1929	3,102,800
1909	16,875,000	1930	2,263,200
1910	21,475,000	1931	No shipments
1911	16,064,005	1932	because of low
1912	13,870,255	1933	rubber prices
1913	4,409,874	1934	891,800
1914	594,334	1935	1,189,600
1915	3,104,047	1936	2,955,500
1916	633,154	1937	7,303,100
1917	2,299,174	1938	5,378,300
1918	4,029,412	1939	6,408,640
1919	2,427,022	1940	10,346,560
1920	2,200,342	1941	11,898,880
1921	64,802	1942	16,163,840
1922	615,770	1943	17,290,560
1923	2,742,779	1944	19,864,320
1924	3,076,200	1945[a]	11,397,120
1925	8,313,200		
		Total	270,824,998

Note: Figures to 1938 by Continental-Mexican Rubber Co.; 1939-45 by E.G. Holt, Rubber Development Corp.
[a]First six months only.

Another result of the revolution was the relocation of the Continental-Mexican Rubber Company from Mexico to the San Diego, California, area in 1912, where 400 acres were purchased and partly planted. At this time the company was renamed the Intercontinental Rubber Company. In 1916 the operation was moved to Continental, Arizona, approximately 20 miles south of Tucson. Unfortunately, neither of these ventures proved profitable.

Between 1912 and 1941, rubber prices fluctuated over a wide range as a result of political and commercial factors. Because *Hevea* rubber (produced by the rubber tree, *Hevea brasiliensis* (A. Juss.) Muell.-Arg.) and guayule rubber are essentially interchangeable in application, the price for *Hevea* rubber also represents the value of guayule rubber. In 1912 guayule rubber sold for $0.48 per pound. In the early 1920s the British restricted the amount of *Hevea* rubber that was exported from Malaysia, and consequently the price of guayule rubber reached a high of $1.23 per pound in 1925. That year the Intercontinental operation moved to Salinas, California, where local farmers were contracted to plant 8,000 acres of guayule. However, in 1931 rubber prices dropped to $0.02 per pound, and at least half of this guayule acreage was plowed under because farmers could not profitably continue to grow it. Due to this drastic price drop, no guayule rubber was imported into the United States from 1931 to 1933.

The economic situation for guayule was so unfavorable at this time that the large 1-million-pound-per-month processing plant at Torreon, Mexico, was closed. Several small plants were built in communities closer to the sources for guayule shrub. Prices began to improve in 1934 because production of *Hevea* was again restricted, this time by a cartel made up of producing countries, and the guayule industry was reactivated on a small scale. About 3 million pounds of guayule rubber was produced from 4,400 acres in the Salinas area between 1934 and 1941, when rubber prices had risen to $0.22 per pound. Throughout the World War II years, an average of 17 million pounds of guayule rubber per year was imported from Mexico by the United States.

During World War II, $45 million was allocated by Congress for the Emergency Rubber Project (ERP). Under this program more than 31,000 acres of guayule were planted in the

western states. About 3 million pounds of guayule rubber was produced by the ERP, as well as another 500,000 pounds processed from native shrub in Texas. During the same period, however, $650 million was spent on synthetic rubber research using oil and coal for feedstocks, and by 1946 synthetic rubber was being produced from oil costing less than $10 per barrel. These prices continued until the early 1970s (Figure 1). Thus by the end of the war the emergence of the new synthetic elastomer industry, along with the availability of surplus stocks of *Hevea* rubber, caused the economic justification for a guayule commercial industry to

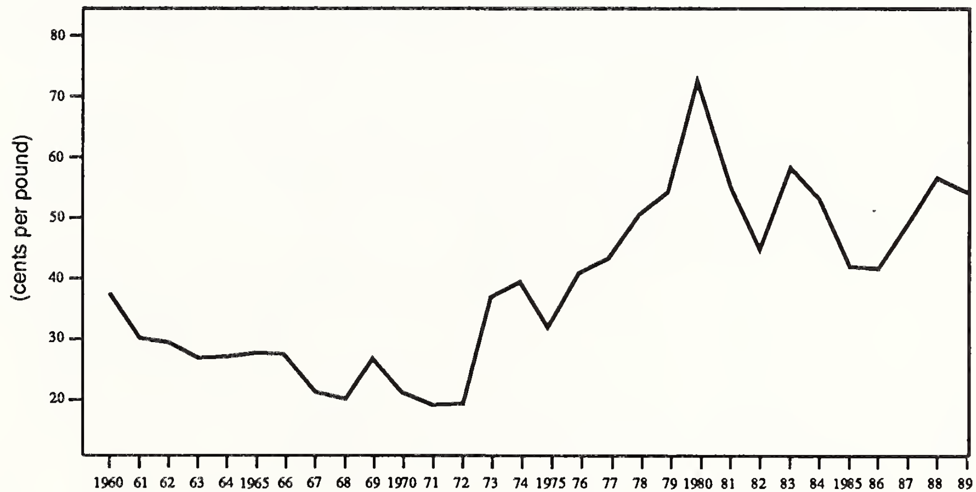

Figure 1. New York natural rubber prices, 1960-89.

disappear, as once more guayule could not compete in price with other rubber sources. In addition, wartime inflation had made field-leasing rates uneconomical for the ERP, and so in 1946 the U.S. Congress ordered the liquidation of all guayule stands and the return of fields to their owners.

After 1946, the USDA continued to investigate guayule but on a very limited scale. In the early 1970s, however, the Arab oil embargo again shifted economic factors affecting the elastomer industries. By 1975, rapidly increasing prices for synthetic rubber feedstock and increased prices for *Hevea* rubber made the costs of guayule production compare more favorably with those of other rubber sources, and thus caused renewed interest in establishing commercial guayule production. Mexico began to explore again the economic potential of guayule in 1974 and determined that 2.6 million tons of wild guayule shrub was available for harvest. In 1976 a small guayule processing plant began operating in Saltillo, Coahuila, Mexico, but no commercial plantations of guayule were established and by the early 1980s this plant had closed.

A U.S. response to the oil embargo was passage of the Native Latex Commercialization Act of 1978, authorizing $30 million for guayule research and development. One of the first projects funded by the Act was to improve the quality of guayule seed available and to increase the amount of rubber that could be produced per acre.

In the early 1980s the U.S. Department of Defense, which had previously investigated guayule in 1930, again became interested in the crop and funded a project to develop a domestic source of natural rubber from guayule. This project had several emphases: to increase seed production, to increase rubber yields, to perfect direct-seeding technology, to determine production costs and economic feasibility of a commercial guayule industry, and to establish the technology and costs of producing "spec grade" rubber from guayule.

Despite the above-mentioned projects, in the last 80 years probably less than 5 percent of the total amount of money committed to *Hevea* and synthetic rubber research has been spent researching guayule rubber production. Consequently, producing guayule rubber on irrigated

land in Arizona using available technology has been estimated to cost approximately twice what it costs to produce *Hevea* rubber (which during 1989 varied from $0.50 to $0.70 per pound). One problem is the amount of guayule rubber that can currently be produced on a per-acre basis compared to *Hevea* rubber. The available guayule cultivars in 1989 are producing an estimated 400 pounds of rubber per acre per year, whereas commercial *Hevea* rubber plantations are producing between 3,000 and 4,000 pounds of rubber per acre per year. Some experimental *Hevea* plots are producing up to 6,000 pounds of rubber per acre per year, compared to the best experimental guayule cultivars, which are producing about 1,000 pounds per acre per year. Thus the amount of gross income that can be generated per acre for *Hevea* as compared to guayule makes the development of a commercial guayule industry appear less attractive economically.

GUAYULE INTO THE 1990s

At present, an economic analysis of guayule rubber production can be no more than an estimate because of a lack of data on guayule shrub production and processing. At the time of writing, there is no commercial-scale guayule processing plant in operation, and the total number of acres currently planted to guayule is less than 800, which must be considered essentially experimental. Thus the calculations presented here are projections only, based on the best information available.

Using the best available data for irrigated and nonirrigated guayule, break-even tables were developed for both types of production. "Break-even tables" show the yields of guayule rubber per acre necessary for a producer to meet costs at various per-pound market prices, and vice versa. It is assumed that high-molecular-weight guayule rubber would be a direct substitute for *Hevea* rubber. Additional assumptions, based on currently available information, are a combined resin and low-molecular-weight rubber (LMWR) value of $0.20 per pound and a bagasse

value of $0.02 per pound. Because no commercial-size processing plant is in operation at this time, the cost of processing the shrub must be estimated. Based on a computer-generated guayule shrub processing plant simulation model, a cost of $0.15 per pound of high-molecular-weight (or "spec grade") rubber is used. Calculations for irrigated guayule were based on a four-year production cycle, with shrub clipped after two years of growth and dug and processed after four years. Under this scenario 400 pounds of high-molecular-weight guayule rubber is produced each year on a per-acre basis.

Total production and harvest costs for irrigated guayule are estimated to be about $700 per acre per year. Table 2 gives the price and yield needed to grow guayule under 1989 conditions and pay all costs (break even). *Hevea* rubber prices (FOB New York) for the spring of 1989 have been in the range of $0.55 to $0.60 per pound. Thus, assuming a rubber value of $0.55 per pound, guayule growers must produce over 1,300 pounds per acre to break even. With the current yield of about 400 pounds of rubber per acre per year, the price would have to be $1.35 per pound to meet costs.

Much interest has been shown in developing guayule as a dryland, or nonirrigated, crop, since it is one crop that has the potential to be grown in the Southwest without supplemental water. The only place in the United States where research indicates guayule could be cultivated under nonirrigated conditions is the "Winter Garden" area of west Texas near Uvalde. The most critical problem facing dryland guayule cultivation, especially if direct seeding is used, is to have adequate moisture to facilitate plant germination and establishment in the spring, which is the preferred planting time. Supplemental irrigation to guarantee seed germination will be necessary in most cases.

Using numbers generated by researchers at Texas A&M University, a break-even analysis was done for dryland guayule production. Total growing costs for dryland guayule are about half of irrigated costs, or slightly higher than $300 per acre per year. Dryland guayule production operates on a five-year cycle (one year more than irrigated guayule), with the plants clipped in year 3 and the complete plant dug in year 5. Some experimental guayule shrub grown from

Table 2. Per-acre income above costs for irrigated guayule production.

		Price of rubber per pound					
		$0.40	0.55	0.70	0.85	1.00	1.15
Pounds of rubber produced per acre per year	200	-425	-395	-365	-335	-305	-275
	400	-385	-325	-265	-205	-145	- 85
	600	-345	-255	-165	- 75	15	105
	800	-305	-185	- 65	55	175	295
	1,000	-265	-115	35	185	335	485
	1,200	-225	- 45	135	315	495	675

Assumptions:
- 1989 dollars.
- Rubber: 400 pounds per acre per year.
- Resin/LMWR: 500 pounds per acre per year (value $0.20 per pound).
- Seed: 0.15 pounds per acre per year (value $150 per pound).
- Bagasse: 6,400 pounds per acre per year (value $0.02 per pound).
- Processing costs: $0.15 per pound of rubber produced.
- Production costs: $700 per acre per year.
- Four-year production cycle.
- Seedlings, irrigated.

transplants in the Winter Garden area has yielded slightly over 240 pounds of rubber per acre per year. Based on the same assumptions for resin and bagasse values and processing cost as given for irrigated guayule, to break even with a 240-pound-per-acre yield, guayule rubber would need to be worth $1.05 per pound (Table 3). At the current price of $0.55 per pound for *Hevea* rubber, a guayule grower would need to produce 600 pounds per acre or 2.5 times the present yield.

Table 3. Per-acre income above costs for nonirrigated guayule production.

		Price of rubber per pound					
		$0.40	0.55	0.70	0.85	1.00	1.15
Pounds of rubber produced per acre per year	200	-167	-137	-107	- 77	- 47	- 17
	300	-147	-102	- 57	- 12	33	78
	400	-127	- 67	- 7	53	113	173
	500	-107	- 32	43	118	193	268
	600	- 87	3	93	183	273	363
	700	- 67	38	143	284	353	458
	800	- 47	73	193	313	433	553

Assumptions:

1989 dollars.
Rubber: 240 pounds per acre per year.
Resin/LMWR: 250 pounds per acre per year (value $0.20 per pound).
Seed: 0.10 pounds per acre per year (value $150 per pound).
Bagasse: 2,600 pounds per acre per year (value $0.02 per pound).
Processing costs: $0.15 per pound of rubber produced.
Production costs: $300 per acre per year.
Five-year production cycle.
Seedlings, nonirrigated.

Preliminary results in the laboratory suggest that guayule resin is composed of several compounds that have the potential to be converted to value-added commercial products, after some additional processing. Using the same assumptions as above but increasing the value of resin to $0.45 per pound changes the break-even points significantly. With rubber selling for $0.55 per pound, irrigated guayule fields would need to produce 900 pounds of rubber per acre

Table 4. Per-acre income above costs for irrigated guayule production (with higher resin/LMWR values).

		Price of rubber per pound					
		$0.40	0.55	0.70	0.85	1.00	1.15
Pounds of rubber produced per acre per year	200	-275	-245	-215	-185	-155	-125
	400	-235	-175	-115	- 55	5	65
	600	-195	-105	- 15	75	165	255
	800	-155	- 35	85	205	325	445
	1,000	-115	35	185	335	485	635
	1,200	- 75	105	285	465	645	825

Assumptions:
1989 dollars.
Rubber: 400 pounds per acre per year.
Resin/LMWR: 500 pounds per acre per year (value $0.45 per pound).
Seed: 0.15 pounds per acre per year (value $150 per pound).
Bagasse: 6,400 pounds per acre per year (value $0.02 per pound).
Processing costs: $0.15 per pound of rubber produced.
Production costs: $700 per acre per year.
Four-year production cycle.
Seedlings, irrigated.

per year to break even (Table 4). This goal is conceivable in light of some of the new guayule cultivars growing in experimental fields during 1989; the best are producing 800 to 900 pounds per acre per year. At the present yield of 400 pounds of rubber per acre per year, rubber would have to be worth $1.00 per pound to break even.

Under dryland conditions, an increased resin value of $0.45 per pound would decrease the

Table 5. Per-acre income above costs for nonirrigated guayule production (with higher resin/ LMWR values).

		Price of rubber per pound					
		$0.40	0.55	0.70	0.85	1.00	1.15
Pounds of rubber produced per acre per year	200	- 92	- 62	- 32	- 2	28	58
	300	- 72	- 27	18	63	108	153
	400	- 52	8	68	128	188	248
	500	- 32	43	118	193	268	343
	600	- 12	78	168	258	348	438
	700	8	113	218	323	428	533
	800	28	148	268	388	508	628

Assumptions:

1989 dollars.
Rubber: 240 pounds per acre per year.
Resin/LMWR: 250 pounds per acre per year (value $0.45 per pound).
Seed: 0.10 pounds per acre per year (value $150 per pound).
Bagasse: 2,600 pounds per acre per year (value $0.02 per pound).
Processing costs: $0.15 per pound of rubber produced.
Production costs: $300 per acre per year.
Five-year production cycle.
Seedlings, nonirrigated.

amount of rubber a guayule producer needed to produce to about 375 pounds per acre per year (Table 5). Under current rubber yields, a dryland guayule producer could also break even if the price of rubber increased to $0.75 per pound.

Break-even Tables 2 through 5 point out the critical need either to increase the amount of guayule rubber produced per acre or to find increased uses of guayule resin and/or other guayule processing coproducts. Some preliminary laboratory efforts to develop value-added products from guayule coproducts are showing very positive results. The low-molecular-weight rubber is being combined with chlorine to form chlorinated guayule rubber, which has several commercial uses; chlorinated rubber using synthetic or *Hevea* rubber has a current market value of over $2.00 per pound. Several other commercial products using guayule resin or low-molecular-weight rubber are going through laboratory tests and are very promising. Because guayule rubber must compete directly with *Hevea* and is tied to *Hevea* prices, it is absolutely necessary to find uses for guayule resins and low-molecular-weight rubber if the industry is to be economically viable and become a competitive supplier for the natural rubber market in the United States.

The Office of Arid Lands Studies (OALS) of the University of Arizona has acquired a computer sensitivity model using Lotus 1-2-3 in which processing plant inputs, costs, and returns can be varied. This model indicates that finding a value-added use for guayule bagasse is extremely important for commercial viability. Because bagasse amounts to about 60 percent of the total weight of shrub entering a guayule processing plant, a $0.01- or $0.02-per-pound increase in its value could make a significant difference in guayule economics. Additional research is needed to try to find added-value uses for this coproduct.

CONCLUSIONS

Although guayule is not currently a commercially viable crop, the economic prospects for a guayule rubber industry are improving. Several new guayule cultivars that have shown early promise and may have the potential to double or triple the existing rubber yields are in the field testing stage. Additionally, improvements in direct-seeding techniques and harvesting methods

will substantially lower production costs. Current research on guayule coproducts points to a potentially profitable industry developed from the resin, low-molecular-weight rubber, and bagasse, and indicates that their value may ultimately exceed the value of the rubber. Thus the coproducts will play a critical role in the commercialization of guayule. When all of these factors have been more fully developed, an economic analysis of a commercial guayule industry will be more accurate and complete.

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Chapter 15

Case Histories of Guayule Production in Australia, South Africa, and the United States[1]

Peter Milthorpe, J.C. Paterson-Jones, J. Wayne Whitworth, George Abel, and William P. Miller

AUSTRALIA

Australian interest in guayule research dates back 60 years, embracing two main periods of investigation; the wartime period of 1942-46 and the past decade. Research into guayule growing has been prompted by different factors at different times. These include filling emergency needs for rubber during World War II and exploring potential export earnings from Australia's extensive dryland farming country. The latest attempt to commercialize guayule was driven by the prospect of dryland production being economic on world markets. The findings of this latter period of intense investigation are of principal concern here.

HISTORY OF GUAYULE RESEARCH IN AUSTRALIA

The historic details of past investigations into guayule research and production in Australia have been adequately summarized elsewhere (1). The early studies attempted to locate areas of

[1] Peter Milthorpe authored the section on Australia; J.C. Paterson-Jones authored the section on South Africa; and J. Wayne Whitworth, William P. Miller, and George Abel authored the section on the United States. References for this chapter are numbered sequentially within each section.

Australia with homoclimes similar to those of guayule's natural habitat in North America. This led to debate as to which areas of southeastern Australia were potentially most suitable.

Consequently, trial plantings were established over vast areas of eastern Australia during World War II when Australian scientists extended the American Emergency Rubber Project (ERP) efforts to Australia. Extremely dry climatic conditions, which prevailed for much of the first half of the 1940s, gave many disappointing results. Hence data now available from this work are scanty and concerned largely with plant establishment techniques for dryland production practices (2). Few data on rubber and resin production were published as most samples had to be sent to America for analysis.

The most encouraging data for dry matter production were obtained from trials grown at Lawes, Queensland, which has high rainfall and rich soils. However, these results were offset by reports of heavy losses of plants due to "wet feet" following heavy rains in 1946 (1).

Interest in guayule then waned until 1980 when the latest investigations into guayule's potential in Australia commenced concurrently with those in North America.

RECENT MOTIVATION FOR AUSTRALIAN RESEARCH

The worldwide escalation of commodity prices following the oil crisis of the mid-1970s renewed interest in establishing guayule production in Australia, particularly with its prospects as an export industry. This interest was fueled by several local factors, which would seemingly enhance the production of guayule in Australia, coupled with optimistic reports of potential production from the United States.

Factors that prompted interest in introducing guayule as a new crop in Australia included the following: the ready availability of vast tracts of relatively cheap, environmentally suitable agricultural land; the easy adaptation of guayule culture to mechanization at all stages of production; and guayule's high value-for-weight and transportability.

Recent cooperative support by U.S. scientists and administrators in the areas of production and supply of seed for trial plantings provided impetus for the initiation of research and development projects. The interest and sincerity in these programs is reflected in the signing of a memorandum of understanding on guayule by the U.S. and New South Wales (N.S.W.) governments in 1982.

CURRENT RESEARCH WORK AND FINDINGS

In 1980, the N.S.W. government, by special funding, initiated an extensive three-year research and development program through the state Department of Agriculture. Simultaneously, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) established an inter-divisional group to draft proposals for future federal research.

These programs subsequently received significant encouragement and guidance from U.S. counterparts. In particular, the N.S.W. program was overviewed by several leading U.S. personnel. During 1980, a feasibility study on the commercialization of guayule in New South Wales was completed (3) and in 1982 a six-person U.S. delegation visited New South Wales and assessed a recently implemented research program (4).

Based on American experience Siddiqui and Locktov (3) predicted rubber yields of 1,100 to 3,300 kg/ha under dryland production with 350 to 500 mm annual rainfall, and indicated that yields toward the high end of their prediction band should be attainable following further research and plant breeding. Guayule production could be shown to be an economic proposition using these yields and rubber prices of A$1.00 to A$2.00/kg.

Both reports contained recommendations to assist the developing N.S.W. research program. Important among these was the need to develop practices for direct seeding and weed control and to obtain data on water use/growth relationships. The need to evaluate the performance of existing U.S. selections in the Australian environment was also mentioned.

The program established by the N.S.W. Department of Agriculture addressed those and other problems. In 1983 a cooperative study between the N.S.W. Department of Agriculture and CSIRO was agreed upon to assess the productive potential of guayule in two different environments. Study sites were selected near Hillston, New South Wales, a dry environment, and at Kingaroy, Queensland, a wet environment. These two sites fall in the broad belt of country in southeastern Australia thought most suitable for guayule culture (Figure 1). Much of this land is currently used for broad-acre cereal growing. The adaptation process to guayule production, as a new complementary enterprise, was deemed to be relatively straightforward.

This band of land has mild climatic conditions during winter, experiencing a frost period of about four months, and dry hot summers. Rainfall is most effective during winter-spring, when soil profiles are recharged, however the northern part has an increasing incidence of summer rainfall and shorter winters.

Frost is not regarded as a problem and snow is nonexistent. Soils vary greatly in type throughout the region with all textural classes present. Areas of sandy, well-drained soils were selected as prime experimental sites but other soils were also planted to guayule.

The results of this recent work are contained in various reports (5-8, 11); a summary of these programs and results follows.

Agronomic Management

Several major agronomic factors controlling guayule growth have been addressed recently. From the data obtained we are better able to predict regions for optimum guayule growth as well as to detail necessary management practices. Soil type and texture are critical and some soils, initially thought suitable, have been found to be unsuited for guayule.

Acid soils. Many sandy-textured and free-draining soils have proven to be entirely unsuitable because of acidity. Soils with a pH of less than 5.0 or with a cation exchange capacity

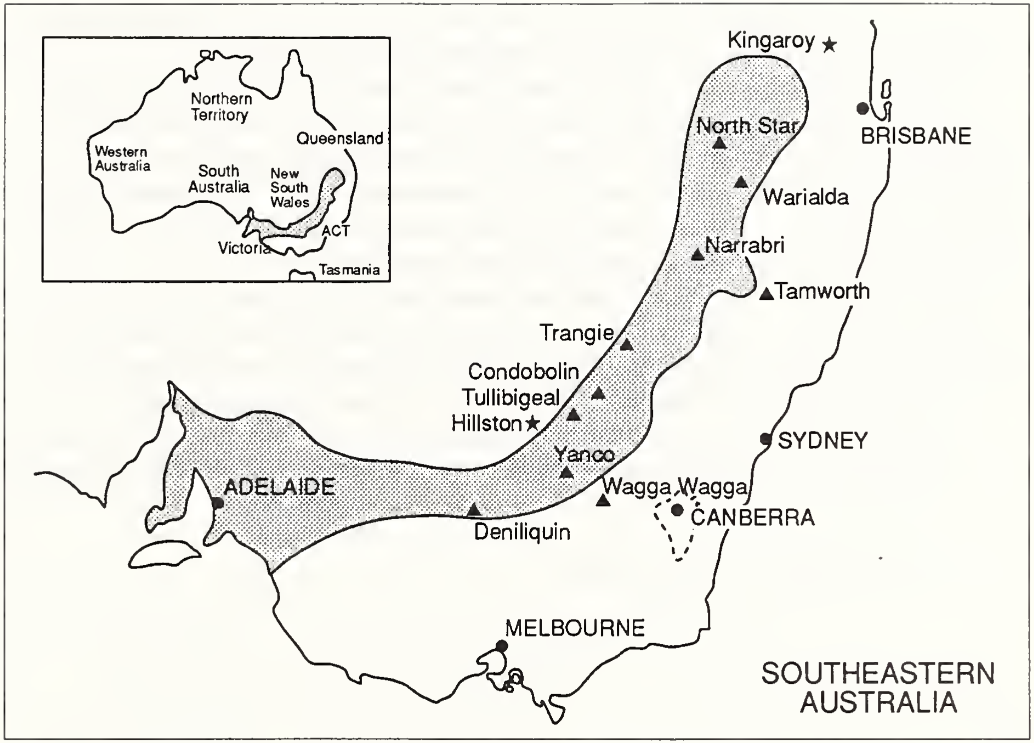

Figure 1. Map of southeastern Australia showing band of country potentially suitable for dryland guayule production.

▲ *field trial sites*
★ *major experimental sites*

containing more than 2 percent aluminum are extremely toxic to guayule. Plants fail to grow and rarely survive more than 12 months, even with good water supplies (5).

Clay soils. Most soil types in the developed irrigation districts of New South Wales have fine-textured topsoils and poor internal drainage. These soils together with the extensive black soil plains of northern New South Wales and southern Queensland have both been found unsuitable for guayule. Episodic prolonged wet periods, a feature of this environment, result in major deaths of plants from root disease. During the past seven years five trial sites located on these soil types have had to be abandoned following wet weather.

Mallee soils. These are soils with sandy-textured topsoils overlying clayey, often calcareous, subsoils. They are usually free draining and are similar to soils in South Australia tested in earlier guayule evaluation programs. Unfortunately most of these soils in New South Wales occur along the western, drier edge of the cereal-growing belt and receive low rainfall.

Red-brown earths. These are the most extensive soils of the cereal-growing belt and appear suitable for guayule, however they may be locally unsuitable due to clay layers near the surface or due to acidity.

Weed Control and Herbicide Evaluation

The land-use pattern, coupled with European ancestral practices, has resulted in the presence of a large suite of introduced, mainly winter-spring growing weeds on much of Australia's agricultural land. Climatically, the best time to establish guayule by direct seeding is during the fall or spring. These times coincide with the periods when weeds are most competitive, thus necessitating the use of machinery or selective herbicides.

The results of herbicide trials indicate that successful stand establishment by direct seeding is not yet possible and that plant establishment will only be achieved by using seedling transplants and preemergent herbicides to address the weed problem. Agronomy professor J. Wayne

Whitworth, New Mexico State University, undertook a detailed program of herbicide evaluation during 1983-84 while on sabbatical leave in Australia.

A large number of herbicides were evaluated for weed-control effectiveness as well as for phytotoxic effect on guayule. Pot studies to test for phytotoxicity were conducted simultaneously with many field trials where pre- and post-emergent herbicides were applied on different soil types, ranging in texture from sands to clays, to test their effectiveness in weed control.

Briefly the findings of these studies were as follows: a) that there is no herbicide suitable for adequate weed control in direct-seeded guayule stands; b) that a mixture of Goal (R) [oxyfluorfen] and Surflan (R) [oryzalin] at the rate of 2.0 kg/ha (a.i.) gives excellent weed control for a full spectrum of weeds for at least six to eight months when applied as a preplant treatment; c) that the use of Round-up (R) [glyphosate] applied as a directed basal spray at the rate of 1.0 kg/ha (a.i.) gives good control of established weeds, especially during the winter dormant period of guayule. However, a significant decrease in growth results if Round-up is applied as an overall spray to guayule irrespective of stage of growth; d) that a mixture of Goal, Surflan, and Round-up applied as a directed basal spray after plantation establishment gives excellent control of establishing weeds as well as having residual effects, and e) that guayule, two years old and older, has excellent competitive ability over weeds and appears to require little chemical or mechanical assistance unless there are prolonged wet periods during its dormancy period.

Plant Establishment

The need for successful establishment of guayule stands by direct seeding has always been of paramount importance and has received a great deal of study, particularly in the United States (refer to Chapter 6). In Australia, where dryland production with commensurate lower yields is envisaged, it was thought that direct seeding would be an essential part of the production technology. Several important local problems had to be addressed apart from understanding the

germination requirements of guayule seed. Studies on guayule seed emergence at the optimum temperature (20-25°C) have shown that at least three consecutive days of rain are required to allow seed to germinate. Following germination there is a further requirement of about six weeks of suitable climate for seedlings to establish. While some of the establishment-period problems can be overcome by fallowing, the probability of receiving favorable germinating rains in the field was found to be extremely low in most years for much of the potential guayule growing area.

Plant Survival and Performance

Trial plots were established at 25 sites in New South Wales between 1980 and 1983 to assess the survival and production of guayule on various soil types and in different environments. Sites were located through a broad band of inland New South Wales (Figure 1) in those cereal-growing areas thought most suitable for guayule. Plot size varied from small (100 plants) to extensive (1 ha). Plant density was generally standardized at 25,000 plants/ha, however, at 3 trial sites guayule was planted at differing densities. Of the 25 sites all but 8 relied entirely on rainfall. The remainder received varying amounts of supplementary irrigation. Limited seed supplies early in the program necessitated the use of "Hansons Bulk" seed to establish 11 sites, however, with the acquisition of seed from selected ERP lines the latter and larger plots were planted with elite, named material.

Several sites were established in southeastern Queensland by CSIRO between 1980 and 1984 using similar seed supplies as the N.S.W. trials. All N.S.W. plots were fallowed prior to planting to conserve subsoil moisture. If dry conditions prevailed at planting seedlings were watered, but if the topsoil was damp seedlings were not watered. Following planting, weeds were controlled mechanically until 1983 after which preemergent sprays were used prior to planting and subsequent weed control was done chemically or in combination with hoeing.

Hardened seedlings were remarkably resilient to dry conditions and all plots, except one, had good survival. Plant losses after three years were generally less than 10 percent. Where losses were greater, they could be attributed to pestilence. At four sites in N.S.W. prolonged wet conditions and associated soilborne disease such as *Phytophthora* spp. were responsible.

Another trial, set up along the guidelines of the regional variety trials conducted in Arizona, California, Texas, and New Mexico, was planted into moist soil in October (spring) 1984 at Hillston. Soon after planting a grasshopper plague swarmed into the area and killed 26.4 percent of seedlings. Despite this and a very dry summer and fall, when practically no effective rainfall was recorded, survival after one year was 67.2 percent, while after three years and just prior to harvest, plant survival was 64.1 percent.

Dry Matter Assessment

The assessment of dry matter production was standardized for all plots and followed the procedure outlined by Nurthen et al. (9). Unless otherwise stated plants were clipped between 7.5 and 10 cm above ground level, dried in a forced-air dehydrator at 50°C for 24 hours, defoliated, and had old flower heads and peduncles removed before weighing and chipping. A sample was taken after chipping and grinding to assess the moisture content of the wood.

Plants to be harvested for dry matter production were selected for trueness of type and bordered by other plants to avoid edge effects. Plots were harvested at different intervals and for different lengths of time depending on availability of material, but most plots were sampled to collect data spanning at least three years.

Production figures were then calculated on 100 percent stands based on the nominal planting density of that trial. All plants harvested at a sampling were bulked prior to drying and weighed to obtain a mean value for that plot.

Dryland production. The production data for a number of dryland trial sites have been selected to illustrate the performance of guayule in New South Wales and Queensland over the past eight years (Table 1). Data have been selected for those sites where three or more years growth has been collected from the top growth of plants.

Dry matter, rubber, and resin production have generally been disappointing at all sites. This may be attributed in part to the extended prevailing dry conditions that occurred during the 1980s.

In New South Wales the wetter sites of Tamworth and Wagga have greater growth for age, however, at Tamworth death of a high percentage of plants following a wet period at 15 months detracts from the productive potential. Two other plots established concurrently at Tamworth on heavy clay soils completely failed during this wet period due to root disease, highlighting the unsatisfactory nature of these soil types. The survival at the Wagga site was good but there was great variation in the performance of the plants from the two different planting times. At the other sites annual production was relatively constant despite site differences. Production from two sites of similar rainfall showed little difference in dry matter production despite soil fertility differences. One site, Tullibigeal, had well-structured basaltic soils of high fertility (90 ppm P) while the other site, at Hillston, had a sandy soil of low fertility (10 ppm P).

The average rubber production per year for most sites ranged between 75 and 120 kg/ha while resin production ranged between 80 and 100 kg/ha. At Tamworth and Wagga rubber production was 220 and 271 kg/ha, respectively, while resin produced was 210 and 189 kg/ha.

At Kingaroy, Queensland, the wettest of all sites, production was highest. At a planting density of 18,600 plants/ha, rubber and resin accumulated at the rate of 295 and 312 kg/ha/y over a three-year period, however, wet conditions during the fourth year seriously depleted this guayule stand (Ferraris, personal communication).

Soil texture did not appear to be influential except for the clay soils where root diseases after wet periods were important. However, soil pH was critical and one trial, established at Narrabri

Table 1. Dry matter, rubber, and resin production from guayule at a number of N.S.W. and Queensland dryland sites.

Site	Average annual rainfall (mm)	Establishment date	Soil type	Line	Age (months)	Rubber (%)	Resin (%)	Production (kg/ha)		
								Dry matter	Rubber	Resin
Warialda L29°32'S, 150°35'E	680	5/81	Sand	H.B.[a]	24	11.44	6.03	2,403	275	145
					29	12.34	8.29	2,820	348	234
					42	11.43	6.05	4,560	521	276
					57	15.61	9.38	4,021	628	377
Hillston L33°29'S 145°32'E	350	9/83	Loamy sand	[b]	12	9.89	9.32	702	69	65
					24	11.96	10.10	1,296	155	131
					36	11.19	8.72	1,811	203	158
					44	10.40	9.34	2,102	219	196
		10/84		11604	38	12.38	8.85	3,878	480	343
				11605	38	13.10	8.87	2,239	293	199
				N565	38	12.42	9.04	3,022	375	273
				11619	38	11.13	7.99	3,227	359	258
				CAL 2	38	4.85	7.02	7,559	367	531
				11591	38	11.58	8.16	2,317	268	189
Wagga L35°4'S, 147°21'E	570	1/81	Sandy clay loam	H.B.[a]	29	5.77	5.44	2,789	161	152
					51	11.01	7.64	11,767	1,117	775
					65	10.89	7.61	13,492	1,469	1,027
		10/82		11591	32	13.35	10.41	5,992	702	544
					46	11.32	10.17	4,975	563	506

Table 1. (continued).

Site	Average annual rainfall (mm)	Establishment date	Soil type	Line	Age (months)	Rubber (%)	Resin (%)	Production (kg/ha)		
								Dry matter	Rubber	Resin
Tamworth L131°5'S 150°51'E	673	9/82	Sandy clay loam	11605	20	6.25	10.65	3,672	391	229
					41	10.93	10.81	6,962	761	752
Condobolin L33°5'S, 147°9'E	439	1/81	Sandy clay loam	H.B.[a]	29	6.20	6.08	1,664	103	101
					48	8.44	7.89	6,127	517	483
					66	8.39	8.07	6,857	576	554
Yanco L34°42'S, 146°30'E	470	1/83	Sandy clay loam	11591	32	11.80	9.20	2,655	313	244
Tullibigeal L33°20'S 146°52'E	400	3/83	Sandy clay loam	11591	13	4.89	6.85	1,230	60	84
					21	8.92	8.48	1,603	143	136
					40	7.36	6.54	4,290	316	293
		6/83		11591	40	10.76	9.15	3,283	353	301
Kingaroy L26°35'S 151°50'E	762	9/85	Clay loam	[b]	12	6.77	—	1,120	75	—
					24	9.87	12.77	3,720	367	475
					36	12.06	12.73	7,356	886	936

[a]Hanson's bulk seed.
[b]Mean value for cultivars N565, 11591, and 11619 planted at 18,600 plants/ha.

on deep sands with a pH of 4.5, failed completely after 12 months despite good rainfall of 500 mm.

Irrigated production. No trial received irrigation equivalent to evapotranspiration demands but rather plots were watered to ensure plant survival and, in some cases, seed set and ripening. The trial at Condobolin, established in September 1981, received frequent waterings for three years but then tapered off to a few summer waterings each year. The second Condobolin site and the Hillston trial received three or four summer-fall waterings each year, approximately equivalent to annual average rainfall, which is 420 and 350 mm, respectively.

Rapid growth occurred at the Condobolin sandhill site over the initial three-year period, but then reached a plateau (Table 2). For some lines, production of dry matter showed an apparent decline in later harvests. Because of limited numbers of plants in each of these lines, harvests taken over the first three years were usually only single plants. However, at the final harvest, five plants of each line were taken. Although plants appeared similar in size there was as much as a twofold difference in stem weights. Rubber production of 350 to 657 kg/ha/y for the first three-year period was achieved, however, the increase in production after this was small. A similar pattern, but at a lower level of production, was achieved at the other sites. This pattern of rapid initial growth followed by a plateauing of production as experienced at Condobolin has previously been noted by other workers (10). The level at which the plateau is reached will be determined by soil attributes as well as the amount of water received annually.

Effect of plant density. Trials established to monitor production of guayule grown at densities between 9,200 and 52,600 plants/ha showed that there were initial increases in production with increasing plant density, but these differences abated with time as the crop approached maturity (Milthorpe, unpublished).

Using a very high planting density (55 plants/m^2) and supplying adequate irrigation, full light interception and a stem yield of 6.9 t/ha was obtained in three months at Lawes, Queensland (near Toowoomba). After one year the stem yield was 11.5 t/ha and plants had a rubber and resin content of 6.9 and 12.6 percent, respectively (11). No further results were given.

Table 2. Dry matter, rubber, and resin production from guayule tops grown under irrigation at Condobolin and Hillston.

Site	Establishment date	Soil type	Line	Age (months)	Rubber (%)	Resin (%)	Production (kg/ha)		
							Dry matter	Rubber	Resin
Condobolin	9/81	Sand	N565	21	6.20	8.40	7,980	495	620
				34	9.00	9.91	11,339	1,021	1,124
				40	13.03	9.73	11,478	1,495	1,176
				59	11.05	8.61	12,725	1,406	1,096
			11591	21	5.39	7.44	4,470	240	333
				34	9.67	8.68	10,924	1,056	948
				59	11.12	9.52	9,195	1,022	881
			11605	21	6.70	9.96	8,405	563	820
				34	9.33	11.46	10,655	994	1,221
				59	11.12	10.52	12,861	1,430	1,353
			11619	21	7.12	10.11	5,468	389	553
				34	10.34	11.21	14,043	1,456	1,574
				40	10.94	10.12	19,886	2,189	2,012
				59	9.85	11.27	13,046	1,285	1,470

Table 2. (continued).

Site	Establishment date	Soil type	Line	Age (months)	Rubber (%)	Resin (%)	Production (kg/ha)		
							Dry matter	Rubber	Resin
Condobolin (continued)	1/83	Sandy clay loam	N565	14	6.68	9.98	3,070	205	306
				27	9.83	10.95	4,254	418	466
				58	10.52	9.97	7,248	762	723
			11591	14	4.64	6.95	5,117	237	356
				27	7.75	7.77	6,304	489	490
				58	8.17	8.39	8,086	661	678
			11619	27	8.74	7.64	3,628	317	277
				58	9.20	8.92	7,592	698	677
Hillston	4/84	Loamy sand	565	13	7.59	9.83	728	55	71
			11591	25	11.20	9.53	1,741	195	166
				37	11.38	9.56	3,675	418	351

Plant variability. At each sampling time plants were selected for uniformity of type and size to ensure that representative samples were taken. Where plant numbers were limited, such as in single-row plantings of ERP material, individual plants, or pairs of plants, were harvested. Phenotypic variation within lines of material is common and where possible "off type" plants were rogued out of the stand or avoided at harvesting. In 1983 Professor J. Wayne Whitworth assessed the phenotypic variability in 160 different plots of 24 ERP lines. He found that there were, on average, about 16.7 percent "off type" plants (Whitworth, unpublished). Some lines varied greatly and the percent of "off types" ranged from 5 to 30 percent. For example, lines N576, N593, N396, and 11600 consistently were true to type while lines N563, 4265XF, and 11701 had a high percentage of "off types." Despite selecting for trueness of type when harvesting, great variation occurred in the weights of seemingly similar-sized plants. When 45 samples of five plants per sample were taken from 10 lines, the mean coefficient of variation was 29.3 percent. ERP lines 12229, 11591, and 11604 were the most variable, while line N565 was the most uniform.

In another sampling of line 11591 after two years' growth, the coefficient of variation in stem weights of a 12-plant sampling was found to be 16.8 percent, reinforcing the findings of Rubis (10) and his recommendations that 10 to 20 plants should be harvested at each sampling, if possible and economically feasible.

Harvesting

Several trials were conducted to see if time of harvest or amount of material harvested affected the survival of the guayule stump and subsequent ratoon growth (4). Results indicate that if some foliage is left on the plant at harvest, the plants will survive irrespective of harvest time. However, if the entire top of the plant is removed, then harvest time is important. Harvesting from the onset of winter conditions until after the break of winter dormancy resulted in little or

no death. In contrast, harvesting from the end of the spring into fall caused significant death of plants. The moisture status of the soil at harvest had a small influence on survival during the summer months. The survival of stumps cut at three times of the year from 1984 to 1986 is shown in Table 3.

ASSESSMENT OF RUBBER PRODUCTION

Several published methods for determining resin and rubber content of guayule were available when this evaluation program began in Australia. Each of the methods required different specialized equipment and varying inputs of labor. There was a need to develop a technique suitable to Australia's conditions that would allow rapid and reliable analysis of resin and rubber in guayule, and hopefully, permit comparison with other research work. In 1983, the proton magnetic resonance (PMR) technique was developed and published by CSIRO (12). Unfortunately this method did not measure resin content and required expensive, specialized equipment. After the evaluation of many of the published methods, the N.S.W. Department of Agriculture decided on a modified soxhlet technique (9). Samples of guayule prepared by this procedure and stored at -18°C were reanalyzed after nine months and the results were un-

Table 3. Survival of guayule stumps cut at Hillston at various times throughout the year.

	Plant survival (%) when top cut		
Year	May	August	December
1984	85.9	85.9	22.4
1985	91.7	96.9	24.0
1986	93.2	99.0	56.8

changed from the initial analysis, indicating that the sample preparation technique and storing facilities were adequate.

The soxhlet method allowed the preparation of samples in the field and their storage before analysis without the risk of degradation. All the samples from the N.S.W. work were analyzed using this method. The method was also used to cross-reference the Queensland work, which relied principally on the PMR technique.

Following their death after the prolonged summer and fall drought, intact dead plants of line N565 at Hillston were tagged in May 1986. They were then regularly sampled and prepared in the standard manner for rubber and resin analysis. The results showed a steady decline in rubber content with time left in the field, together with a corresponding steady increase in resin content (Table 4).

Table 4. Change in rubber and resin content of dead guayule (line N565) left standing in the field.

Harvest date	Time dead (months)	Rubber (%) Dry weight	Resin (%) Dry weight
May 86	2	9.41	8.68
Aug 86	5	7.08	9.66
Dec 86	9	7.15	11.77
May 87	14	4.51	10.39
Aug 87	17	6.19	10.29

Rubber Quality

No assessment of quality of the rubber produced under Australian conditions has been attempted. The modified soxhlet method of analysis does allow the translucent, amber sample to be collected after solvent evaporation but lack of facilities has precluded any further evaluation of rubber quality.

ECONOMIC APPRAISAL OF GUAYULE IN AUSTRALIA

No economic appraisal of guayule has been carried out in Australia using locally collected field data. However, several studies have been completed where projected yields and costs have been applied. Siddiqui and Locktov (3) in 1981 completed a study showing that guayule would be economic if yields of 1.12 to 3.36 t/ha of rubber over five years could be achieved and the price of rubber held between A$0.88 and A$2.20/kg. A later study by Rawlins (13) based returns on a rubber yield of 8.4 t/ha (from direct-seeded material) over a six-year crop cycle, with harvests after three and six years using a price of A$1,260/t for rubber.

No dryland trial results have approached the projected yields of rubber quoted in those studies. Additionally, experience has shown the need to implement the costly practice of using transplanted seedlings to establish guayule stands under dryland conditions. From the added costs of supplying seedlings together with the smaller returns from the low rubber yields it is unlikely that dryland guayule will ever be economical at current rubber prices.

SOUTH AFRICA

Guayule was first planted in South Africa during World War II when seed was sent here by the United States Department of Agriculture. Some plants were established at an agricultural experiment station in a small town, Fauresmith, in the Orange Free State. Two of these plants or their offspring survive there still.

In 1978, a private company, Envirotech (Pty.) Ltd., asked the University of Stellenbosch to investigate guayule in South Africa. Professor E.W. Laubscher visited Tucson that year and brought back from the University of Arizona the first guayule seed. This was germinated at the Welgevallen Experimental Farm in Stellenbosch to provide the first plants for experimentation.

In 1979 the author was appointed to start and manage a collaborative program (1, 2) of research on guayule administered by the Cooperative Scientific Programmes unit, now incorporated in the Foundation for Research Development of the South African Council for Scientific and Industrial Research (CSIR). Research groups that have contributed to the program are listed in Table 5.

The aim of this program is to determine the practical and commercial feasibility of growing guayule as a local source of natural rubber for the South African rubber manufacturing industry. Its motivation is the local supply of a vitally necessary industrial material—all natural rubber used in South Africa currently is imported. A second objective is the provision of a crop for marginal agricultural areas that have been badly affected both by the recent drought and by national surpluses of conventional crops like maize.

Since 1979 the program has benefited from regular contact with researchers in the United States and elsewhere, through the membership of South Africans in the Guayule Rubber Society (GRS) and their attendance at international guayule conferences and GRS meetings. The assistance the program has had from these contacts is gratefully acknowledged.

Table 5. South African Guayule Programme—participating research group.

Group leader	Affiliation
Professor E.W. Laubscher	Department of Agronomy and Pastures, University of Stellenbosch, Welgevallen
Professor J.J. Human/ Mr. M.H. du Plessis	Department of Horticulture and Agronomy, University of the Orange Free State, Bloemfontein
Professor E. Graven	Department of Agronomy, University of Fort Hare, Ciskei
Dr. J.T. Meynhardt	Vegetable and Ornamental Plant Research Institute, Roodeplaat, Pretoria
Mr. E. Wicht	Department of Agriculture, Winter Rainfall Region, Stellenbosch
Dr. R.D. Sanderson	Institute for Polymer Science, University of Stellenbosch
Professor F. Hugo	Department of Mechanical Engineering, University of Stellenbosch
Professor J. Van Staden	Department of Botany, University of Natal, Pietermaritzburg
Professor J.H. Visser	Department of Botany, University of Stellenbosch
Professor T.A. Modro/ Dr. J.C. Paterson-Jones	Department of Organic Chemistry, University of Cape Town

THE CLIMATE AND SOILS OF SOUTH AFRICA

The Republic of South Africa occupies the southern tip of Africa between the lines of latitude 22°S and 35°S. Within its borders lie several independent countries such as Bophuthatswana and Lebowa (Figure 2). Figure 3 shows the distribution of total annual average rainfall. The northwestern part of South Africa is arid and includes part of the Kalahari Desert; there is a general increase in rainfall from west to east. The eastern coastline, together with small areas of high rainfall inland at the edge of the Drakensberg escarpment, has the highest rainfall in the country. The monthly distribution of rainfall also varies from west to east with a winter rainfall pattern in the west, an intermediate region of no pronounced seasonal maximum, and the largest region of predominantly summer rainfall, which includes the areas of highest precipitation.

In general, South Africa has an equable climate, without great extremes of temperature or humidity (3). Humidity in the summer rainfall areas in summer is moderate in the inland areas but high on the east coast; humidity is low inland in winter in this area. Table 6 presents average daily

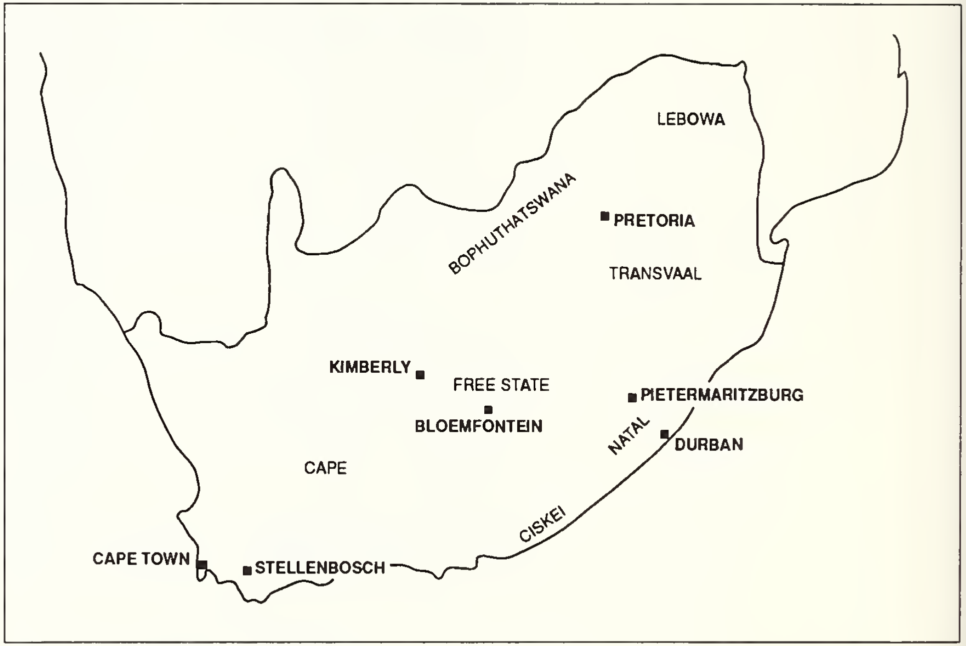

Figure 2. South Africa—regions and cities quoted in the text.

Table 6. Average daily maximum temperatures in the hottest month, average daily minimum temperatures in the coolest month, and average daily temperature range in the coolest month.

Location	Avg. daily max. temp. —hottest month (° C)	Avg. daily min. temp. —coolest month (° C)	Avg. daily temp. range —coolest month (° C)
Cape Town	26.3	6.9	10.5
Kimberley	32.5	2.8	15.9
Bloemfontein	30.5	-2.1	19.5
Pretoria	28.1	3.2	16.0
Durban	28.0	10.0	12.7

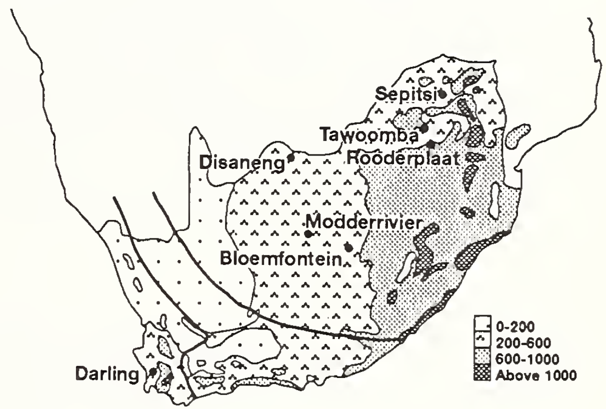

Figure 3. South Africa—rainfall distribution and trial sites. The thick lines separate winter rainfall, all-year rainfall, and summer rainfall areas. Four regions are shown with annual average rainfall in mm of 0-200, 200-600, 600-1,000 and above 1,000. The major trial sites are named and small adaptation trials are shown as small dots. The cross-hatched areas are envisaged for guayule cultivation.

maximum temperatures in the hottest month, average daily minimum temperatures in the coolest month, and an average daily temperature range for the coolest month for a variety of locales. As expected, the greatest daily and seasonal temperature differences are experienced inland; these temperature differences are very important for the cultivation of guayule with a high rubber content.

Guayule requires a neutral or alkaline calcareous soil. In South Africa, deep, fertile calcareous soils are not widespread (4) but there are large areas east of the Kalahari and Botswana with red sands or sandy loams on a limestone underlay, some of which are suitable for guayule. The sands and sandstones of the southwestern areas are highly leached, infertile soils and are unsuitable. The more heavily structured soils on lime are suitable but limited in extent. The central plateau region known as the Karoo has some suitable soils but is generally too arid, and in many areas, the soils are saline. Most conventional crop production in South Africa is in the north-central, northeastern and eastern regions of summer rainfall (the northeastern Free State; central, northern, and eastern Transvaal and Natal) on soils of medium to high fertility. Wheat is also produced on shales in the southwestern Cape.

Very large areas of South Africa are uncultivated and used for low-income, extensive production of sheep or cattle. Borderline areas have been used unreliably for crop production, the success in any year depending on the amount of precipitation locally. Social and economic problems in these marginal agricultural areas have been made worse by the recent drought (1982-86), which stopped the production of conventional crops.

POTENTIAL AREAS FOR GUAYULE CULTIVATION

The areas in South Africa envisaged for the cultivation of guayule are indicated in Figure 3. These are areas with suitable soils (neutral to alkaline, calcareous, deep sandy loams to loams, without excessive salinity) with low to moderate rainfall (350-600 mm total/y in the summer)

and low winter minimum temperatures. Guayule has always been regarded essentially as a dryland crop in South Africa. The use of scarce irrigation water for an extensive, low-income crop like guayule is not considered a high priority.

THE CULTIVATION OF GUAYULE

Seed

Seed of the standard USDA varieties was obtained from several sources in the United States and Israel. The pioneer work of germinating this original seed, the establishment of foundation seed plantings, multiplication of the seed and its storage, and initial work on the production of transplants for adaptation trials and agronomic experiments was carried out at the Welgevallen Experimental Farm of the University of Stellenbosch. Seed of some newer varieties such as A101 was later added to the seed collection. Currently there are some 3 ha of foundation plantings at Welgevallen containing the varieties 11609, 11591, 11604, 11605, 12229, 12231, A-48118, N-563, N565 II, N-593, 11634, 11635, N-566, N-565, N-575, N-576, 11600, A-48124, A-48121, A-48143, N-396, 11701, 11619, 4265FX, and A101. No diploid guayule is planted out in the field.

Transplant Production

Transplants for outside trials and experiments were initially produced at Welgevallen in the form of hardened-off, topped, bare-rooted plants of three to nine months old, following the method used in the United States World War II Emergency Rubber Project. Establishment rates in the field using this material were erratic and often poor, as low as 50 percent in some cases. Damage to the transplants' roots during lifting led to fungal infection after planting out. The

generally low establishment percentages achieved with this type of transplant certainly also had to do with the drought conditions prevailing in the test areas at the time of planting the trials. In some areas, the soils were dried out to a depth of 2 m, and since guayule was to be considered a dryland crop, only a limited amount of water (1/2 l/plant) was given to each plant immediately after planting out, without any further irrigation being supplied.

The poor results achieved with this type of transplant, admittedly under abnormally dry conditions, have led to the development of an automated production of "speedling" type transplants, which have given more reliable establishment. This system was developed at the University of the Orange Free State.

Establishment

In the summer rainfall areas, establishment of transplants has been most successful in fall when rainfall has reestablished soil moisture and temperatures are moderate. In the winter rainfall region, establishment in spring has proved the most successful for the same reason.

Adaptation Trials

Initially it was thought that the western Cape coastal region would provide suitable conditions for the growth of guayule and rubber production. This is a winter rainfall area (Figure 3) with warm, dry summers. It was thought that drought stress in summer would provide good conditions for rubber production.

Several small trials were established in this region. Within two years it was clear that guayule was totally unsuited to this type of climate except where the winter rainfall was enough to allow soil moisture to build up to a level that allowed a long period of growth in spring when ambient and soil temperatures were sufficiently high. Even in the optimum winter rainfall areas, growth was limited. Small adaptation trials were established in suitable summer rainfall

areas in 1981 (Figure 3). It was rapidly clear that the combination of rainfall and warm temperatures provided the correct conditions for the growth of guayule. The finding that cold, dry winter conditions led to rubber production in guayule (see section on Bioregulators, Plant Anatomy, and Plant Physiology later in this chapter) confirmed that these conditions were optimum for growth and rubber production in guayule.

Towoomba Trial

Although plants in all the adaptation trials have been assayed for growth and rubber content, this data could only be taken as a very preliminary indication of potential yields. More reliable data have been obtained from a trial at Towoomba, north of Pretoria. This trial, established in March 1981, consisted of one 50-m double row of plants of each of the cultivars 11591, 11604, 11605, 12229, 12231, A-48118, N-563, N565 II, N-593, N-566, and N-576 planted with the following spacing: 100 cm in the row, 70 cm on the diagonal. Four harvesting procedures were tested: a) a tops only harvest at two years old with the same plants harvested completely after five years; b) a tops only harvest at two years old with the first complete harvest at three years old; c) a tops only harvest at two years old with the first complete harvest at four years old; and d) a complete harvest at five years old. The best yield results, expressed in g rubber/plant, are shown in Table 7 and were obtained from plants harvested only once, whole at the end of the trial.

Agronomic Experiments

Researchers at Roodepat have conducted experiments to determine the best weed killer for use with guayule, to determine the best season for planting out in the summer rainfall area, to optimize the age of transplants, and to determine yields.

Table 7. Best cultivar yields at Towoomba.

Variety	g rubber/plant (average of 4 plants)
11605	124
11591	105
A-48118	97
N-565 II	89
N-563	87
N-593	86

The yield trials are not yet complete. Guayule will be planted at a density of between 20 and 40 thousand/ha from nursery-grown transplants. The optimum density from direct seeding has yet to be determined. A major determinant of plant spacing is the cost of plants; direct seeding can economically provide far greater plant densities if sufficient seed is available.

Propagation. The physiology of seed formation and germination has been investigated (5) and guidelines have been set for the production, harvesting, treatment to counter germination inhibition, germination of guayule seed, and the production of healthy viable transplants (6). Some success has been achieved with the propagation of guayule by cuttings (7).

Mycorrhiza. Vesicular arbuscular mycorrhiza have been found (8, 9) to promote the growth of small guayule plants in South African soils in which phosphorus is a limiting nutrient. Since many soils in South Africa and particularly those in the potential growing areas for guayule are deficient in phosphorus, this finding has an important potential application. The economics of inoculating transplant material are being considered by Professor Human's group and the practical improvement of guayule's growth in the field after inoculation is being tested.

Pests and diseases. In some early trials using bare-rooted, topped transplants, poor establishment was achieved because of a wilt disease, which probably entered the plants through wounds in the roots; the responsible pathogen was tentatively identified as a *Fusarium* species. Occasional deaths of established plants in fields seem to be the result of similar infections, but these do not pose a significant problem in the soils used for planting guayule in South Africa.

No major insect damage has been noted on guayule plants in the field.

In preliminary tests, cattle and sheep avoided grazing guayule but goats grazed guayule in the absence of any other grazing.

Weed control. "Goal" is a safe and useful preplanting and general weed control agent for use with guayule.

Current Agricultural Research

In the initial phase of this program we learned:

- how to handle guayule as a horticultural subject;
- how to produce, clean, treat, and germinate seed and produce plants for the field;
- the best potential growing areas for guayule in South Africa;
- the pitfalls in establishing this dryland crop;
- how to manage the crop in the field; and
- something about the yields to be expected.

It has become clear that guayule has potential in certain areas in South Africa, but before a definite conclusion can be reached about guayule's commercial viability here, definitive data on the costs of growing guayule and on rubber yields in the potential growing areas are needed. The thrust of the second phase of agricultural research on guayule is the following. Trials of 3.6 ha were planted at the time of writing (October 1988) in three localities: Bloemfontein,

Disaneng in Bophuthatswana, and Sepitsi in Lebowa. It is hoped that these trials will generate definitive data on yields and growing costs as a function of age at harvest, harvesting practice (multiple, single), rainfall, population density, fertilization, and cultivar. The first results of these trials are expected in September 1991. Seed supply will be the critical factor in the expansion of guayule cultivation through pilot plantings to full-scale commercial plantings. Because of this, established seed lots at Modderrivier (1/2 ha each of 11591, 11604, 11605, 11619, A-48118, N-565, N576, 12229, and A101) were scheduled for expansion in Bloemfontein in April 1989 to 2 ha and in November 1989 to 4 ha. These seed lots were under irrigation to maximize seed production.

In the United States, direct seeding is being researched as a low-cost method of establishing guayule. The research there aims at rapid germination in the field or the planting of pregerminated seed. Enormous amounts of irrigation water (up to 2,000 mm) are then required to keep the emerging seedlings alive and to prevent salt accumulation. This approach is not feasible for establishing essentially dryland guayule as envisaged for South Africa. Direct seeding clearly offers the same economic advantages here, and research on establishing guayule under dryland conditions using direct seeding is being done at the University of the Orange Free State. The aim of this research is direct seeding without irrigation using rain-harvesting techniques. The germination of guayule in the field generally presents no problems. The survival of the seedlings and their competition with other plant growth provide the major challenges. In fact, although in the floods of 1987-88 some plants were submerged and drowned at Modderrivier, on adjoining higher ground very large numbers of volunteer seedlings appeared that year because of the higher than normal rainfall. The survival rate of these seedlings is being monitored. Volunteer seedlings have appeared before in dryland culture here and at other trials; some of these have survived to maturity. No evidence of the formation of retoños (suckers) has been found in South Africa.

Direct seeding is regarded here as research for the future. We realize that direct seeding is only going to be possible as a commercial means of establishing guayule once commercial-

scale plantings are producing seed, since direct seeding uses vastly more seed than the nursery production of transplants. It follows that the first commercial-scale plantings will have to be established using nursery-produced transplants to make optimal use of scarce seed.

Bioregulators, Plant Anatomy, and Plant Physiology

To strengthen the depth of knowledge about guayule and to provide support for the growing of guayule to produce rubber and seed, Professor Van Staden's group at the University of Natal built up research expertise in the anatomy and physiology of guayule and factors governing growth, seed production and, in particular, rubber formation. At the same time, the idea that a guayule cell culture might provide an easier system than the whole plant in which to study rubber biosynthesis led to the establishment of a project on the cell culture of guayule under the leadership of Professor Visser at Stellenbosch University. Work on the synthesis of bioregulators and their testing has been carried out by Professor T.A. Modro and the author at the University of Cape Town.

An early success was the demonstration of an effective defoliation of guayule using 2-chloroethylphosphonate (Ethrel, Ethephon), which produces the senescing hormone, ethene (10, 11). Extensive ultrastructural studies using electron microscopy have contributed to the understanding of where (in which cells), how, and under what physiological conditions guayule produces rubber and what the role of specific cells and organelles in this process is (11-20). Concurrently, studies on the production and transport of photosynthates and their metabolism for growth or for rubber production under different environmental conditions have clarified the conditions required for the rubber-production process (21-24). The development of a novel bioassay (25, 26) for determining the current rubber-producing potential of any individual plant without destroying it has allowed a detailed investigation (19, 22, 27) into environmental effects and seasonality on growth and resin and rubber biosynthesis in guayule.

The response of different guayule cultivars to different day lengths and day/night temperature regimes has been studied (28-30). Under winter temperature conditions (15° C day/ 4° C night), variety 12231 produced more biomass under long days than short days whereas variety 11591 showed the reverse. In an experiment to determine the effect of the duration of cold treatment on rubber production, 11591 yielded more rubber under short days than long, whereas 11604 produced more rubber under long days.

The establishment of a viable suspension cell culture of guayule has proven difficult (31-36) and although the problems have largely been overcome, the cultivars are slow growing and do not provide an easy system in which to study rubber biosynthesis. Apart from this aspect, plants have been regenerated from cell cultures and, together with success in protoplasting guayule cells, there is the possibility of creating high ploidy guayule plants through protoplast fusion.

An improved synthetic method has been developed to produce the bioregulator 2-(3,4-dichlorophenoxy)ethyldiethylamine (DCPTA) and analogous compounds (37, 38). This structure has been incorporated in a chemical structure designed to improve absorption in mature plants (39) but, in fact, a later study (40) of the rate of absorption of the dimethyl analog of DCPTA showed that it is rapidly absorbed by guayule and transported throughout the plant. In several tests on guayule plants in which the short-term effect on rubber production of DCPTA or analogs was tested (2), no practically significant increase in rubber content was found—on the contrary, DCPTA at 5,000 ppm concentration sprayed on nine-month-old plants in summer killed them. A definitive test on the short- and long-term effects of DCPTA on guayule growth and rubber production is under way in Pietermaritzburg.

Coproducts Research

An investigation of the composition of guayule resin (the acetone extractables) (41) was followed by a search for industrial uses by the Polymer Research Institute at the University of Stellenbosch. Some promising applications include wood surface treatment and stabilization by

whole resin and the nonsaponifiable components, termite protection by whole resin, and a novel application of the whole resin as a binder for tarmacadam road-repair material. This last application is currently being evaluated. The essential oil from the leaves did not find a ready potential market in industrial perfumery. No work has been done in South Africa on the coproduct bagasse since it is assumed that this will form the energy source for the rubber and resin extraction process.

Rubber Extraction

Rubber extraction has not been investigated in South Africa. Current technologies for process development in a pilot-scale precommercialization phase (i.e., Saltillo, Bridgestone/Firestone, Texas A&M) could be used in South Africa. Given the current state of the industry, ample time would also be available to develop alternative technologies.

Economics

South Africa produces no natural rubber at present; styrene-butadiene and butadiene rubbers have been produced locally for many years and in 1984, local production of synthetic polyisoprene started. Despite intense promotion, the synthetic polyisoprene has replaced only 40-50 percent of the previous usage of natural rubber and the rubber manufacturing industry has imported a relatively constant quantity of *Hevea* rubber annually since 1984. This is despite the protection of the local production of synthetic polyisoprene by an import duty of 25 percent on *Hevea* rubber.

In 1979, when the South African Guayule Programme started, the exchange rate of the South African Rand to the U.S. dollar was approximately R1.00 to US$1.25; at the time of writing (October 1988), the exchange rate is R1.00 to US$0.40. This enormous drop in value

has resulted in a corresponding increase in the price of imported commodities priced in U.S. dollars such as natural rubber. This, together with the import duty, has raised the price of commonly used grades of *Hevea* rubber to over R4.00/kg. If this situation persists, the potential for the establishment of a South African natural rubber agro-industry is great. This is in contrast to the economic situation in the United States in which, with the yields realized from currently available strains of guayule cultivated under irrigation on expensive land, the production of guayule rubber appears uneconomic in comparison with the importation of *Hevea* rubber.

In 1986, the Industrial Development Corporation (IDC), a development bank, evaluated the cost of producing guayule rubber in South Africa. Assuming that the value of the coproduct resin would offset resin and rubber extraction costs, the IDC study indicated profitability (12 percent return on investment) at a selling price of R2,500 per metric ton for the rubber with a yield of 2,000 kg rubber/ha over a six-year growing cycle (initial tops-only harvest after four years and a whole-plant harvest two years later). At 20,000 plants/ha this implies 100 g rubber per plant or at 8 percent rubber content, a total biomass of 1.25 kg dry weight per plant after six years. These figures can be attained with the varieties currently available. The Towoomba trial yield figures ranged from 61 to 124 g rubber/plant for several different varieties. The Towoomba yield figures were for whole plants harvested after five years of growth under drought conditions. In this trial, the plants were widely spaced (13,300 plants/ha population) rather than the minimum accepted population of 22,000/ha and, because of the technique used for establishment (bare-rooted topped seedlings), poor establishment (57-84 percent depending on variety) was obtained. For an acceptable 95 percent establishment at a population density of 22,000, the rubber yield/plant figures translate into 1,338/ha for the poorest variety to 2,592 kg/ha for the best. The figure of 2,000 kg/ha over a six-year growth cycle used in the IDC study appears reasonable. For an average rainfall of 600 mm/year, this implies 6.7 kg rubber/ha/cm rainfall. A figure of 5 kg/ha/cm rainfall has been achieved for dryland guayule culture in southern Texas using the standard varieties. Initial trials of newly developed varieties such as

CAL-6 and CAL-7 indicate increases in yield of between 60 and 100 percent over the standard varieties.

The recent large increases in the landed cost of *Hevea* rubber in Rand terms have improved the potential profitability of guayule rubber production in South Africa; the IDC is currently revising its costing to incorporate the latest yield and cost data.

The Future of Guayule in South Africa

The present phase of the South African Guayule Programme with major CSIR involvement will end in 1991-92 with the production of the first data from the yield trials. Negotiations with the rubber manufacturing industry, agricultural development companies and the chemical engineering industry for the continuation of the program into a pilot phase have made it clear that industrial adoption depends strongly on the perceptions of these industries (42, 43) of the long-term performance of the South African economy; guayule rubber production will have to be profitable to be viable. Local economic factors may make it so.

UNITED STATES

GILA RIVER INDIAN COMMUNITY GUAYULE PROJECT

In October of 1981 the Amerind Agrotech Laboratories (ATL) were established by the Gila River Indian Community (GRIC) in Arizona to pursue new crop research, development, and commercialization (1). Hereafter, ATL/GRIC will be referred to as GRIC.

In September of 1982 GRIC was awarded a contract with the NAVAIR, Department of Defense (DOD), which provided for a loan to develop by 1988 a "Prototype Domestic Natural Rubber Industry" based on guayule. Subcontracts were let to Bridgestone/Firestone (formerly

the Firestone Tire and Rubber Company), the Dravo Corporation, and the American Indian Consultants to develop a feasible processing, evaluation, and testing prototype system. The GRIC project provided several initial factors: first, 19 ha of USDA lines of guayule established in November of 1981 along with seed of these lines and of Mexican bulk; second, an established research arm with access to land and laboratory facilities; and third, an established planting of 3,000 plants of a "double large" guayule selection that came to be known as Gila. GRIC proposed to establish a 20-ha Gila seed nursery by 1983 and plantings of this selection for rubber production in the amount of 130 ha each year, 1984-87. The first two plantings were to be established by transplanting and the last four by direct field seeding. GRIC cautioned in their proposal that although preliminary results had indicated that their Gila selection of guayule was superior to the other USDA varieties at 10 months of age, there was no guarantee that at the end of two years of growth it would meet projected rubber yields. Also, since no prototype processing plant had been built, it was possible that their best efforts might not lead to an economically viable method of processing guayule rubber.

The method of loan repayment proposed by GRIC was based on the assumption that Gila would be superior and high-yielding and investors would pay a premium price to obtain the seed. GRIC assumed seed sales would be sufficient to repay the loan (1). It was also envisioned that GRIC would have patents or would share patent rights for a guayule planter, harvester, baler, and seed-cleaning equipment. The processing subcontractor would also have patented their processing technology.

Modification of the Contract

As the anticipated income from Gila seed sales and patent rights did not materialize, it became apparent that it was not possible to pay back the loan funds while research and development were still continuing. The contract and subcontracts were modified to continue as a firm fixed-price contract beginning on September 12, 1986, and ending on December 31, 1988. This

contract called for maintenance and harvest of 111 ha of guayule shrub, construction of a prototype processing plant, and processing of shrub into 51 metric tons of natural rubber (2, 3). Active participants in this project included Bridgestone/Firestone; Dravo Engineering Companies, Inc.; and GRIC. Four southwestern land-grant universities (located in Arizona, California, New Mexico, and Texas) agreed to provide guidance when asked to do so (2, 3).

THE GILA SELECTION

History and Early Promise

The story would not be complete without an understanding of the origin of, the promise of, and the performance of the Gila guayule selection. It originated from a large, vigorous off-type plant found in a seed increase block of the USDA variety 11591 that was planted and maintained by the University of Arizona. GRIC personnel obtained seed from this plant in 1980 with the hopes that its apparent double biomass production would result in a doubling of rubber yields over the USDA varieties (1). This seed collection gave rise to the Gila selection and another seed collection from the same plant by breeders from the University of Arizona gave rise to AZ-101. Isozyme marker studies ("fingerprinting") indicated that these two germplasms are apomictic progenies of a single plant (Chapter 4).

Gila yields after 10 months of growth were reported as 215 g of biomass for an individual plant (dry wt) versus 75 g for a USDA variety, 11591. Rubber yields for the same were 15.4 g versus 7.8 g, respectively. The method of analysis was not reported but the percentage rubber content averaged 7.9 versus 10.6, respectively (1). Both values are inordinately high, especially so for that age of shrub.

A contingency plan was developed in the event that the projected biomass of 1.4 kg per plant and 12 percent rubber were not realized when the Gila variety was again sampled at 24

months. The plan called for utilizing available seed of USDA varieties N396, N556-II, 12229, and 11591 to establish 162 ha of shrub by transplants. Ongoing harvests of seed from these varieties were projected to supply 313 kg of seed by 1984 and that would be sufficient to plant 5,585 ha in 1984 (1).

Performance

In his 1985 quarterly assessment of the progress of the project, Captain Ronald Graves reported the results of analyses of the Gila line for the October-December 1982 period (4). Gila plants at 8, 10, 14, and 18 months of age were reported to have rubber contents of 4.4, 8.1, 1.9, and 2.1 percent, respectively. Branch samples taken in March-July 1984 of three-year-old Gila plants were analyzed by GRIC and by Bridgestone/Firestone. An average of four samples taken at different dates showed 8.0 percent by the GRIC analysis versus 8.4 percent by Bridgestone/ Firestone. Analyses of rubber content by the same labs differed for a branch sample from a 11591 plant, 11.3 versus 9.5 percent, respectively. During this same period, Bridgestone/ Firestone reported on a whole-shrub analysis of Gila plants of different ages. Rubber content at nine months, one year, and three years of age was 0.5, 4.0, and 6.0 percent, respectively, and biomass weights were 110, 410, and 4,154 g/plant, respectively (5).

In his review of the seventh quarterly report, April-June 1984, Graves noted that GRIC recognized errors in their blender method of rubber extractions and analysis (4). They noted that branch sampling did not give an accurate evaluation of total plant yield. Reporting on 2,000 individual plant analyses of Gila two-branch samples from shrubs six months old and three years old, they noted rubber contents that varied from 0.4 to 16.3 percent. In April 1984 GRIC began using the Hammerstrand solvent extraction method of analysis of branches from single whole plants. The wide variation between branches of a single plant accounted for suspected errors (6).

As late as October 4, 1985, the general manager of the project cited a rubber content of 9 percent in 1985, and a projected content of 10-12 percent by 1991 as crossing and selection improved the line (5). In documents furnished by GRIC to members of a technical agronomic team on October 23, 1985, a value of only 5.0 percent rubber (average for Gila plants 2.8 years old) was used to compute rubber yields (see Table 8). A letter from Bridgestone/Firestone in April 1988 indicated that the highest total rubber content in Gila shrub (ages 27 to 47 months) was only 3.5 percent, with an average of only 3.2 percent. On a usable rubber basis only the planting made in June 1983 met the minimum requirement of 2 percent. Usable rubber percentage was calculated as equal to total rubber content x [(0.019 x Mooney viscosity) - 0.39] (7).

Included in Table 8 are values for USDA lines that show that they exceeded Gila both in percentage total rubber and in yield of usable rubber. Because the plants differ in age of shrub when sampled, Table 9 has been included for a more fair comparison of the two germplasms. Unfortunately, the Gila line was not included in this regional variety test due to the proprietary nature of the Gila variety. However, AZ-101, a relative of Gila, was included in the test and produced a rubber content of only 2.8 percent. The highest total yield for Gila plantings at 38 months of age was 741 kg/ha of rubber with a rubber content of shrub of 3.2 percent (Table 8). This is considerably less than the highest USDA line with 1,147 kg/ha at 36 months of age and a rubber content of 5.4 percent (Table 9).

A comparison of the 3.5 percent rubber in 33-month-old Gila plants (Table 8) with the 2.8 percent rubber in 36-month-old AZ-101 (Table 9) would indicate that breeding and selection by GRIC may have made some improvement in the original Gila line. Both of these germplasms started from the same mother plant and AZ-101 has undergone little or no reselection or improvement attempts. GRIC reported in 1988 that a single plant of Gila, G9-21, sampled at the age of three years, showed a rubber content of 5.9 percent and a biomass yield of 45,696 kg/ha with a rubber yield of 2,683 kg/ha (8).

GRIC may also have made progress in the areas of cold and disease tolerance by saving and propagating G9-21 and other selections that survived killing frosts and severe infestations of

Table 8. Sampling of Gila selections for productivity two to four years after early estimates made in October 1985.

Gila plantings No.	Date	Age when sampled (months)	Area planted (ha)	Sampling Report of Sept. 1988[a] Shrub (kg/ha)	Rubber (%) Total	Rubber (%) Usable	Rubber (kg/ha) Total	Rubber (kg/ha) Usable	Early estimates[b] Total rubber (kg/ha)
2	10/83	47	7.7	22,051	3.1	1.5	684	331	878
5	9/84	38	12.6	23,146	3.2	1.8	741	416	1,154
7	3/85	33	5.7	13,798	3.5	1.5	483	207	878
10	7/85	27	13.8	21,325	2.8	1.9	597	405	1,907
USDA:									
Bulk	10/81	72	6.9	15,791	6.5	2.8	1,027	442	NA[c]
12229	10/81	72	1.6	15,680	6.6	4.4	1,034	696	NA
Mexican:									
Bulk	10/81	72	10.5	15,825	7.1	4.1	1,124	649	NA

Total Gila	39.9 ha	Total Gila usable rubber	14.3 metric tons
Other	19.0 ha	Other	11.0 metric tons
	58.9 ha		25.3 metric tons

[a]Reference letter to Bill Cole, Bridgestone/Firestone, from Richard Wheaton, Director of the Office of Critical Materials, USDA, dated September 7, 1988, and citation (10).

[b]GRIC report of October 23, 1985. Tonnage varies as stands varied and computations were based on the yield per plant times the number of plants/ha. An average rubber percentage of 5.7 percent was used based on documents furnished by GRIC to the Technical Agronomic Review Team on October 23, 1985.

[c]Not available.

Table 9. Guayule regional yield test results from the third-year harvest in six locations.[a]

Line	Content (%)		Yield (kg/ha)		
	Rubber	Resin	Biomass	Rubber	Resin
CaL-6 (C250)	6.2 a	8.0 abc	21,931 a	1,332 a	1,818 ab
CaL-7 (C254)	5.7 a	8.0 abc	22,299 a	1,291 a	1,834 ab
11604	5.4 a	7.5 abc	20,688 a	1,147 abc	1,634 ab
11634	5.6 a	8.2 ab	19,537 a	1,095 ab	1,651 ab
N396	6.2 a	6.9 bc	17,387 a	1,019 abc	1,651 ab
11605	5.4 a	7.8 abc	18,187 a	900 bc	1,437 ab
N576	5.4 a	6.7 c	17,705 a	884 bc	1,208 b
AZ101	2.8 b	8.4 a	25,005 a	650 c	2,155 a

[a] Average values from six locations (Arizona, California, New Mexico, and three locations in Texas). Four replications in each location. Values within each column followed by the same letter are not significantly different at the 5 percent level by Duncan's multiple range test.

Macrophomina phaseolina (9, 10). Whether the new selections also have an improved content of usable rubber (high molecular weight) is yet to be determined by further analysis. Indeed, the same may be said of other new, high-yielding lines such as CAL-6 and CAL-7 developed by California (Table 9).

The samplings taken in September of 1988 indicated that the Gila plantings would fail to meet early estimates of usable rubber and could not meet the total of 51 tons required by the contract. Even when combined with the GRIC plantings of USDA lines and Mexican bulk, the total of 58.9 ha could produce only 25.3 tons (Table 8).

Seed Production, Harvesting, and Processing

The original contract projected that enough Gila seed would be collected during the period 1983-87 to plant the required area of 130 ha/y and to stockpile 22,700 kg of threshed, clean seed by the end of the period (1). Seed in this discussion refers to an achene in which threshing and cleaning have removed the attached bract and pair of dry male florets.

GRIC estimated that 1 kg of threshed, clean seed could be produced from 20 kg of unthreshed Gila seed—a conversion factor of 5 percent. Experience by other states indicated that this value was optimistic. New Mexico reported a range of 0.4 to 8.8 percent, and the average from this state, along with three others, ranged from 2 to 3 percent (11).

The first seed collection from the mother plant of Gila was made by GRIC in 1980. This 29 g ultimately gave rise to 3,000 transplants. Seed harvested from these 3,000 G1 plants in 1982-83 produced enough transplants to establish 17 of the 20 ha planned for the production of more seed. As of December 31, 1987, GRIC reported a seed inventory equivalent to 142 kg of usable Gila seed (using their calculated value of 5 percent threshability) (12). This amount of seed would produce enough transplants to establish 760 ha according to GRIC. The inventory also reported 6 kg of seed of USDA varieties. Seed requirements had been drastically reduced when in 1985 it was decided not to sell Gila seed because of its proprietary nature (13). Also the change from direct field seeding to greenhouse-produced seedlings further reduced the need for large amounts of seed (see the section on Plant Establishment). This change was forced by direct field-seeding experiments failing to show success.

Seed was harvested with a cotton harvester modified to a four-row self-propelled guayule seed harvester. Threshing was done with a 152-cm air pressure thresher. Cleaning the seed required some development work to achieve a product essentially free of trash. Pentane was used to float off empty seeds.

Plant Establishment

In 1982 GRIC proposed using transplants to establish the first 130 ha in 1984 and direct field seeding for 130 ha each year thereafter for 1985-87. By 1982, independent researchers in four states, using limited seed supplies that were available, had attempted small direct-seeding experiments with more failures than successes. Previous large-scale plantings by seed in 1951 had been relatively successful. The USDA in their Guayule Seedling Stockpile Project used high rates of seeding to achieve a 44 percent success rate when 210 ha survived of the 467 ha that were direct field seeded (14). A seeding rate of 4.54 kg/ha was used to produce an ultimate stand of 20 plants/m of row (15). This rate, equivalent to 1,191 seeds/m of row, was used because the viability of the seed was sometimes less than 40 percent (personal observation, J.W. Whitworth). Abandonment of some of the fields was due to one or more of the following factors: heavy rains, hail, blowing sand, insects, poor soil types, excessive summer temperatures, and weed competition by weeds resistant to selective seed oil sprays (14, and personal observation, J.W. Whitworth).

GRIC had disappointing results with a number of small direct-seeding trials of 1 ha or less. Some initial tests in June of 1981 by GRIC gave a stand of 10 plants/m from a seeding rate of 66 seeds/m of row—an emergence and survival rate of 15 percent (1). Additional experiments failed due in part to adverse weather, insects, blowing sand, or residual problems with herbicides (6, 9, 10, 12).

Based on the amount of seed sown, GRIC reported a success rate of 6 percent in a small direct-seeded experiment conducted in the spring of 1987. This compares to success rates of 5, 6, and 28 percent reported in 1983 by Whitworth (16) and to 65 percent reported by Bucks using conditioned seed (Chapter 6). The 65 percent was based on a count 30 days after seeding and dropped to 34 percent after two months. GRIC reported on a very successful experiment of 1.2 ha conducted in October of 1987 where the rate of seeding was not mentioned, but initial

stands 15 days after seeding were 82 plants/m of row. Three months later the stand diminished to 26 plants/m—a survival rate of 32 percent (12).

Direct field seeding is not as dependable a method as transplanting for establishing guayule fields and requires considerably more seed. On a 97-cm (38-in.) row spacing, a seeding rate of 164 seeds/m of row, as suggested in the original contract, would require 0.7 kg of seed/ha (using a count of 992,070 seeds/kg). The same amount of seed with a viability of 70 percent could theoretically produce enough transplants for 20.4 ha assuming GRIC's requirement of 23,712 plants/ha (with an allowance of 1,050 plants as an overage for discard and planting loss). Their targeted stand was based on a plant spacing of 46 x 97 cm (18 x 38 in.).

Conversion of seed to transplants is somewhat less than 100 percent efficient. When producing over 500,000 guayule transplants, two California nurseries posted the following conversion rates: 80 percent and 40 percent (16). This would compute to enough transplants from 0.7 kg of seed (73-94 percent viability) to transplant 10.9 ha in the first case and 6.5 ha in the second. These values were achieved by precision planting of well-cleaned, highly viable seed into greenhouse flats. GRIC was unable to achieve this and developed a value for their operations of enough transplants produced from each 0.7 kg of seed to set out 2.4 ha of guayule.

As no commercial nursery chose to remain in the guayule seedling and transplant business when the contract was in force, GRIC had to rent and renovate, and finally buy kits and erect their own greenhouses. In spite of all these difficulties, they were able to establish 17 ha of guayule for seed production by October of 1983. Although this was a little short of their target of 20 ha, it was quite an achievement under the circumstances. USDA varieties were transplanted to 3 ha in 1983. Subsequent transplanting operations established 45 ha of Gila in 1984, 51 ha in 1985, 7 ha in 1986, 4 ha of Gila and 15 ha of USDA varieties in 1987, and 9 ha of Gila and 6 ha of USDA varieties in 1988. A total of 133 ha of Gila and 24 ha of USDA varieties were established by transplants in the six-year period from September 1982 through June 1988 (6, 7, 8, 10, 12). Plant populations ranged from a density of 10,379 to 39,538 plants/ha. About half of the fields approached the target stand of 22,662 plants/ha (7). A plant population experi-

ment showed a trend for higher rubber yield/ha as stands increased from 12,356 to 61,778 plants/ha (8).

Harvest and Delivery of Shrub for Processing

During the first quarter (October-December 1986) of the second contract, GRIC delivered 8 metric tons of whole Gila shrub to the Shrub Preparation Facility in the San Tan Industrial Park on the Gila River Indian Reservation in Sacaton. Balers were tested for densification of shrub and a self-propelled forage chopper was also evaluated for harvesting and densification of shrub. Bridgestone/Firestone determined that too much degradation would occur in the chopped shrub, so GRIC delivered baled shrub for processing (16).

Ground-breaking ceremonies for the construction of the Guayule Rubber Prototype Processing Facility were reported in the third quarterly report (April-June 1987) (10). The opening of the facility officially took place on January 14, 1988, and information given there indicated the facility would have a capacity of 12 tons of shrub/day. Bridgestone/Firestone was responsible for the design and construction of the plant and for its first nine months of operation.

During the sixth quarter (October-December 1987), 5 tons of baled shrub were delivered to Bridgestone/Firestone after considerable difficulty with the one-ton big baler (12). The next delivery (January-March 1988) of 69 tons consisted of both whole and chopped shrub. A used Heston Field Queen No. 7600 Forage Harvester was used to chop most of this delivery. The tying fingers located in the compress chamber of the one-ton baler were susceptible to damage and left bale unbound. The one-ton baler was replaced with an OMC 596 Series II Roll Baler, which, when harvesting the much larger Gila variety, continued to experience breakage of the spring metal tines. In some instances the broken metal fingers were in the bale and caused breakdowns in the processing plant. The stripper bars had to be fabricated of thicker metal. During the period April-June 1988, another delivery was made in the amount of 55 tons bringing to 129 tons the total amount delivered for processing (7). From this and possibly other deliveries, 6 tons of processed rubber was produced (Chapter 11).

On April 13, 1988, Bridgestone/Firestone's director of technology wrote a letter to the Procurement Division of the USDA Office of Operation noting that only the non-Gila shrub and the Gila shrub from the first planting (June 1983) met the specification of 2 percent minimum usable rubber, that all the other shrub designated for delivery failed to meet this minimum and that the total quantity of specification-grade shrub available for delivery was estimated to be no more than 416 tons—far short of the 2,057 tons set out in Schedule H-8 (d)(2)(b) of the contract (7). Consequently, the goal of 51 tons of usable rubber also could not be met. Fields were selected from GRIC plantings that would yield about 25 tons of usable rubber from 58.9 ha and the balance of the goal of 51 tons would be nearly met by harvesting seed increase blocks of guayule planted under government sponsorship by land-grant universities in Arizona, California, New Mexico, and Texas. Samplings of these blocks indicated a possible yield of 22 tons of usable rubber from 21 ha planted to USDA varieties in 1981 (see also Table 6 of Chapter 11).

SUMMARY AND CONCLUSIONS

Goals set by GRIC in the original contract were optimistic. The establishment of 17 ha of the Gila seed nursery by 1983 nearly met the goal of 20 ha. Since all the seed for this originated from a single plant selection made in 1980, this was no small achievement. GRIC was unable to establish plantings of the Gila selection for rubber production in the target amount of 130 ha each year of 1984-86. The 1984 planting was to be established by transplanting and the 1985-87 plantings by direct field seeding. Since no reliable methods of direct seeding were developed, greenhouse-produced Gila seedlings were used to transplant 45 ha in 1984, 51 in 1985, and 7 ha in 1986.

The Gila selections did not live up to expectations, and the yield projected for two-year-old shrub of 1.4 kg biomass/plant (dry wt) with a rubber content of 12 percent was never realized because, in part, the Gila original projected rubber yields were based on the assumption that all

the rubber processed would be usable rubber. However, research by Bridgestone/Firestone showed, for the first time, that all guayule varieties contained a percentage of nonusable rubber, thus lowering the usable rubber percentage in all guayule varieties (1). Theoretically, a stand of 22,662 plants/ha of such plants would produce a rubber yield of 3,807 kg. Actual yields produced by 38-month-old Gila plantings were at best 741 kg/ha of total rubber and only 416 kg of this amount was of high enough molecular weight to be classified as usable rubber (Table 8). This compares with a yield of a USDA variety, 12229, of 1,034 kg/ha total rubber and usable rubber of 696 kg/ha, or with a total rubber yield of 1,147 kg/ha for three-year-old shrub of the USDA variety 11604 (Table 9). Therefore, the GRIC substituted the USDA lines for Gila.

Some improvement in the Gila line was accomplished by reselection and there was an increase in rubber content from 3.5 to 5.9 percent in an advanced selection, but the 12 percent level was never achieved. Nor were any selections of Gila at any time impressive enough to attract a market for the projected price of $1,542/kg of seed.

On September 12, 1986, a modified contract was signed that called for maintenance and harvest of 111 ha of guayule shrub, construction of a prototype processing plant, and processing of shrub into 51 metric tons of natural rubber. Three years later, the prototype processing plant had been built, some of the 111 ha of guayule shrub had been harvested and processed, and 6 of the 50 targeted tons of natural rubber had been produced by the processing plant. Some difficulties were experienced with the processing plant and at the time of writing (September 1989) the biomass feed rate capacity of 996 kg/ha (fresh shrub rate) had not been reached (Chapter 11).

No attempt has been made to minutely detail the problems GRIC experienced nor their development of methods and equipment to overcome them. Successes in the areas of seed harvesting and cleaning, greenhouse production of transplants, transplanting operations, plant breeding, irrigation, shrub harvesting, and processing may be measured by the results that are briefly reviewed in this chapter.

The experience of the GRIC is a valuable one and future endeavors will do well to consider this experience as they attempt to develop an economically viable processing facility. Until a new variety of guayule is developed that exceeds the break-even level, or rubber prices more than double, it will be difficult to interest private growers and investors in a new rubber industry based on guayule.

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Chapter 16

Future Research and Development

Marvin E. Jensen

INTRODUCTION

Guayule, like many other plants, has a long history of use as a source of raw material. Guayule is native to North America and may also have been found in South America. The native habitat of guayule occurs in areas of limited rainfall, calcareous and well-drained soils, high summer daytime air temperatures, low nighttime temperatures, and mild, dry winters. Guayule competes and survives in this environment because it is extremely drought tolerant. One of its drought-tolerant characteristics is its deep root system that, on deep well-drained soils, enables it to access water not available to other plants. Another drought-tolerant characteristic of guayule is its ability to become partially dormant under conditions of water stress and to recover rapidly when water becomes available (Chapter 9).

The extraction of guayule rubber differs greatly from *Hevea* rubber extraction. Guayule rubber forms as deposits within cells; *Hevea* rubber occurs as a latex in plant ducts. Thus, guayule plant material must be harvested and processed to extract rubber whereas rubber trees are tapped to extract latex. Rubber trees continue to grow after latex is extracted. Guayule plants that have been harvested by clipping must regrow, or, if whole plants were harvested, the plants must be reestablished. This difference between *Hevea* and guayule plants has had major economic effects on guayule natural rubber production.

Forces Affecting Guayule Rubber Development

Because a reliable source of rubber was needed during World War II, the Emergency Rubber Project was established in 1942 to develop a domestic source of rubber from guayule. When the war ended, this incentive faded, and most of the guayule research resources were diverted to other, higher priority research needs. All development was terminated.

Rapidly rising oil prices in the 1970s stimulated a renewed interest in developing guayule as a source of natural rubber and associated by-products. Research conducted during the 1940s was not complete, and there were significant knowledge gaps in some aspects of guayule growth and development and in processing technology. Promoters of guayule commercialization made claims that could not be substantiated or refuted by available research data. Unrealistic projections of potential yields were made, such as those for dryland areas in Australia (Chapter 15). Guayule, a drought-tolerant crop, was promoted as an alternative crop for water-short irrigated areas. Such claims were unrealistic and received unwarranted attention. Erroneous claims also provided ammunition to those who opposed allocating scarce research resources for guayule development.

Knowledge Gaps and Deficiencies

Guayule research conducted during the past 15 years has provided extensive data to fill knowledge gaps and expand the knowledge about guayule growth, rubber production, and processing technology. For example, detailed experiments have clearly shown that guayule is very drought tolerant, but, as with all plants, water is consumed in producing biomass. If water limits plant growth, biomass production is related to the amount of water available in a given environment.

In arid areas, potential transpiration rates are higher than in semiarid areas. Detailed studies showed that rubber yield depended on the amount of irrigation water applied and consumed.

The amount of rubber that could be produced per unit of water consumed with current lines ranged from 0.04 to 0.05 kg/m^3 (110 to 135 lb/ac-ft) under both dryland and irrigated conditions (Chapter 7). Competition for limited water resources in the western United States is increasing and water costs are escalating in many areas. Under current rubber prices and water costs, the net return per unit of water consumed by guayule in most arid, irrigated areas makes it an unattractive alternative crop.

During the late 1970s claims were made about the potential increases that could be achieved in the rubber content of the guayule plant through plant breeding. Earlier reports of rubber contents ranged from 3.6 to 22.8 percent. Detailed field trials with old and newer lines in several environments produced data sets that clearly showed that rubber content and rubber yields are conservatively low under both dryland and irrigated conditions. Controlled field experiments showed that rubber contents of old and current lines ranged from 3 to 8 percent (Chapter 4). Recent research also showed that the production of rubber per unit land area could be increased, but increased production was achieved mainly by increasing the amount of biomass produced. Increased biomass production also meant increased harvest and shrub transportation costs.

Interdisciplinary Research and Communications

The recent guayule research effort, though very limited, has been unique in that guayule researchers have established national and international communications in all aspects from plant breeding to plant processing. In the United States, a joint informal USDA-State Agricultural Experiment Stations "Guayule Administrative Management Committee" (GAMC) was formed to coordinate guayule research. The GAMC appointed a researcher as technical coordinator for both state and federal guayule research. Many research projects involved interdisciplinary teams. From the mid-1970s, researchers from many disciplines and countries met periodically to exchange data and discuss research results. The recent research effort has

been unique in that guayule researchers from many disciplines learned to communicate with each other to expand the overall guayule knowledge base.

Sharing germplasm was not as successful as sharing information. Delays were encountered in obtaining newly developed lines, which were reported to have very high rubber contents, for field trials. When these lines were obtained, planted, grown, harvested, and sampled for rubber content and yield under standard procedures, the ranges in rubber contents and yields were much smaller than had been expected based on previous claims.

Future Guayule Research Programs

Policymakers and managers of scarce research resources now face a dilemma. Where do we go from here? Shutting down a research and development program and restarting it again delays progress. Retraining scientists, reestablishing communication linkages, and repeating some research to regain practical experience in response to another crisis is *not* good management of research resources. Will there be a future payoff for continued investment in guayule research and development? There are benefits from continuing an integrated, interdisciplinary research effort versus continuing only a narrow discipline-oriented program or restarting an interdisciplinary effort when another crisis becomes eminent.

Many issues need to be addressed. If the current economics of domestic guayule rubber production does not become competitive with imported natural rubber produced from *Hevea*, there must be some justification for continuing a research effort, such as enhancing national security. Natural rubber is a strategic national defense material. Guayule rubber production by the private sector must be economically productive to be viable. Today, in Australia and the United States, with current lines and prices, guayule production is not economically viable. Local economic factors can make significant differences in the economics of guayule rubber production with current technologies. For example, the large decline in the value of the South

African rand relative to the U.S. dollar has resulted in economic conditions in South Africa that differ greatly from those in the United States and Australia (Chapter 15).

As oil resources are depleted and oil costs rise, will guayule rubber become economically competitive with other crops, and, if so, when? Who should fund a continuing research program? How large should this effort be? Who should be responsible for managing the research program? How should a reoriented program be implemented to maintain a viable, stable, and productive research effort? These are policy issues that need to be addressed. Policy issues cannot be discussed constructively and policy decisions cannot be made rationally without a reliable database. Continued research at some level is needed to produce and update the guayule database as new technologies in plant breeding, chemistry, plant growth modeling, and processing become available. Research breakthroughs are not inevitable, but breakthroughs can occur that may lead to large gains in production technology.

The purpose of this chapter is to briefly summarize research needs that were identified by guayule researchers and authors of prior chapters to assist in research planning, setting priorities, allocating resources, and implementing programs. The key decision that must be made is whether the United States should establish a goal to commercialize guayule as a reliable, domestic source of natural rubber. The views expressed are those of the author. They have been modified and tempered by comments and suggestions from several reviewers.

CURRENT TECHNOLOGY—BRIEF OVERVIEW

Germplasm Resources

A general summary of progress made in guayule breeding and in current production technology is presented in Chapter 4. Studies have shown that guayule plants may have 36, 54, 72, or a higher number of "A" chromosomes. Different chromosome numbers make guayule a difficult

organism to work with in terms of breeding. Current plant breeding and genetic research is concentrated at Riverside, California, and at Tucson and Phoenix, Arizona. Estilai and Ray (Chapter 4) identify possible sources of erroneous rubber yield projections. Projected rubber yields based on individual plant rubber yields and plant densities generally were not realistic because of interacting effects. Similarly, projections of water use by individual plants under laboratory conditions were not realistic because, under field conditions, guayule does not respond much differently from other plants grown under similar soil, water, and climate conditions.

An understanding of the natural rubber chemistry also is essential in developing markets for guayule coproducts. New insights into the inheritance of rubber quality and biochemistry of rubber synthesis have evolved from the recent research efforts (Chapter 2).

Similarly, much has been learned about the leaf morphology and stem anatomy as they relate to rubber content. These are parameters that breeders use to select new plants.

Rubber Formation and Quality

We know much more today about the quality of guayule rubber compared with *Hevea* rubber than we did 15 years ago. We know that the quality is comparable to that of *Hevea* and that quality is affected by many factors including post-harvest degradation of rubber quality. However, we have not learned much about the factors that affect post-harvest degradation.

Environment-Production Interactions

Rubber formation appears to be cyclic and may be stimulated by low, nonfreezing temperatures. Low night temperatures appear to induce expression of genes that code for the enzymes involved in rubber synthesis (Chapters 3 and 5). High-molecular-weight rubber is produced

during winter periods and low-molecular-weight rubber is produced during rapid growth periods.

During the past 30 years, much has been learned about crop growth and water stress, crop yield and water consumed, and total water requirements for most agricultural crops. The research conducted during the past 10 years indicates that guayule responds to water stress like most other crops. Many of the same principles apply to guayule. When water is available, guayule will consume as much or more water daily than even alfalfa because of its low canopy resistance and its aerodynamic characteristics, which affect the transfer of sensible heat from the air to the plants under arid conditions (1). The primary source of energy for evapotranspiration is solar radiation. A secondary source is sensible heat transferred from the ambient air to well-watered plants whose leaf temperature is less than that of the air.

The relationships of water stress relative to rubber yield, to water and plant nutrient requirements for plant establishment, and to clipped and whole plant yields are summarized in Chapter 7. Water stress-yield relationships have also been determined, and the water-use efficiencies of various lines under both dryland and irrigated conditions have been summarized.

Yield functions versus climatic variables, including the formation of low- and high-molecular-weight rubber, are summarized in Chapter 9. Guayule is more salt tolerant than previous reports indicated. Nakayama (Chapter 9) concluded that irrigation is one of the best management tools and that a guayule plant simulation model could be used to assess probable production under various climate and management regimes.

Improved practices have been developed for producing nursery seedlings and preparing them for transplanting. Seed cleaning, storage, and treatment technologies have been developed that have increased the probability of success in establishing guayule by direct seeding (Chapter 6). Direct seeding can reduce establishment costs if high plant densities are required.

Plant Health

Guayule is a hardy plant, but nursery-grown plants are subject to many diseases. A detailed review of pathogenic and beneficial microorganisms affecting guayule seedlings and plant pests is presented in Chapter 8.

Harvesting, Packaging, and Transport

Significant improvements in seed and shrub harvesting have been made and these are summarized in Chapter 10. Most of the studies indicate that guayule shrub should be processed soon after harvesting to avoid degradation in rubber quality. Recent research has adapted modern harvesting technology, including densification and transportation, to guayule shrub harvest.

Extraction, Processing, and Utilization

New and improved procedures have been developed for preparing feedstocks for processing. Experiences with several improved extraction methods are summarized in Chapter 11. Several pilot processing operations have been developed and guayule rubber has been processed for testing purposes. Improved techniques have been developed to extract both rubber and resins. Low-molecular-weight rubber has been eliminated from high-molecular-weight rubber in recent processing demonstrations. Currently, coproduct applications are limited, but benefits from coproducts may offset a major part of processing costs (Chapters 12 and 13).

Some guayule rubber performance tests have been completed. Applications to date indicate that guayule rubber is an acceptable substitute for *Hevea* rubber. Coproduct applications are limited, but benefits from coproducts can exceed processing costs.

Economics

Under current prices, the cost of producing guayule rubber in irrigated areas is about twice the cost of producing *Hevea* rubber. Break-even production tables have been developed for both dryland and irrigated conditions (Chapter 14). Costs on dryland are about one-half those on irrigated land. The authors concluded that rubber yields must be increased or other uses for the resins must be established for guayule to compete under present prices. Milthorpe (Chapter 15) concluded that it is unlikely that guayule will ever be an economic crop in Australia at current rubber prices.

Prototype Guayule Production-Processing Industry

In an effort to develop a pilot guayule production and processing unit in the United States, the Naval Air System Command, Department of Defense, provided a $20-million loan to the Gila River Indian Community (GRIC) to develop a prototype domestic rubber industry based on guayule by 1988. In 1982, a contract was awarded to the Amerind Agrotech Laboratories (ATL), which had been established by GRIC in 1981. Several subcontractors were involved including Bridgestone/Firestone (formerly the Firestone Tire and Rubber Company). ATL's proposal relied on their Gila selection of guayule, which very preliminary testing results indicated was superior to existing USDA-certified cultivars at 10 months of age. This included speculation that the line would be so attractive that growers would pay $1,540/kg ($700/lb) to obtain its seed. The proposal also envisioned developing and obtaining revenue from patents on planting, harvesting, seed cleaning equipment, and processing technology (Chapter 15).

In retrospect, the probabilities of success in each of these efforts, and especially in "finding" a new line among off-type plants that was so much better than lines that had required years to develop, meant that the overall probability of success was very low. Samples of the Gila line in

September 1988 had significantly lower rubber contents than USDA lines and other existing lines. The combined results of this effort are summarized in Chapter 15.

The GRIC-Department of Defense effort made clear the importance of having a reliable research and production performance database on which to base major development analyses and decisions.

RESEARCH AND DEVELOPMENT NEEDS

In October 1983, the Guayule Administrative Management Committee sponsored a workshop for guayule researchers and administrators to identify guayule research needs and a five-year plan. A total of 18 objectives were devéloped at the workshop. The most important objectives in order of priority for the 1984-88 period were:

1. Develop new cultivars with high yield and rubber quality.
2. Develop more economical methods of plant establishment for both irrigated and dryland guayule production.
3. Define rubber quality, develop standard analytical procedures, and define mechanisms of post-harvest rubber deterioration.
4. Develop processing technology for irrigated and dryland practices to maximize economic yield for clipped and whole-plant harvesting.

Significant progress was made toward these objectives during the following five-year period, and many of the results have been summarized in the previous chapters. In 1987, a task-force committee updated the list of research objectives for 1988-90. The updated list contained five goals, which are summarized here (2):

Goal 1. Develop guayule germplasm for higher rubber yield and quality with desirable growth, agronomic, and processing characteristics.

Goal 2. Develop an understanding of molecular biology, biosynthesis, and bioregulation for increased guayule natural rubber production.

Goal 3. Develop and demonstrate improved agronomic methods for guayule plant establishment, cultural management, harvesting, and crop-residue disposal.

Goal 4. Develop improved processing technologies for guayule natural rubber, resins, and high-value coproducts.

Goal 5. Determine the economic feasibility for guayule commercialization in the United States.

During the past three years, significant progress has been made toward several of these goals. Some aspects of guayule research, such as determining water requirements, guayule plant responses to water stress, seed and shrub harvesting methods, and production economics based on performance data, do not need to be conducted on a continuing basis. Research approaches used to determine many of these responses are similar for many crops as is the adaptation of processing technologies. Therefore, comprehensive studies under several of the above objectives need be initiated only after sufficient new guayule lines have been developed. Other aspects of guayule, such as germplasm development, require a continuing program to avoid lost time under start-stop operations.

Production-User Organizations

The regular meetings of guayule researchers have provided valuable exchanges of research data and have established important communication linkages. As guayule rubber production increases, users of natural rubber need to learn more about the special characteristics of guayule rubber relative to *Hevea* rubber. Similarly, if and when private guayule growers become involved, even on a limited scale, an association of guayule growers and processors of guayule shrub needs to be established to permit communication and exchange of ideas concerning problems and alternative approaches to solving or adjusting for problems.

PLANNING AND IMPLEMENTATION

A key factor affecting future guayule research is whether or not officials and administrators will agree to a national goal of commercializing guayule as a domestic source of natural rubber. Guayule rubber production currently is not economically competitive with *Hevea* rubber and will not be competitive in the near future. Therefore, a national goal of commercial production of guayule rubber is essential if a viable, productive research program is ever to exist. Achieving progress toward an economically competitive guayule rubber industry will require a stable research and development guayule program.

Goals and Objectives

The goals and objectives of a guayule commercialization program should involve maximizing returns from the allocation of limited research resources to guayule development. This will require a strategic plan with clearly defined goals, objectives, and priorities. Resources must be allocated proportionally to those areas that have the largest potential for payoff. Critical analyses of potential payoff from alternative approaches will be needed.

Before deciding to allocate funds to a particular areas of research, a variety of factors must be considered. For example, the potential exists for transferring salt and cold tolerance from the species *Iva frutescens* L. to guayule (Chapter 2). But developing cold tolerance in guayule may not be a high priority given the following facts. It appears that low, nonfreezing temperatures stimulate rubber deposition (Chapter 3), but that rubber production per unit area is highest when daytime temperatures are high because of greater biomass production. In the large areas of the United States that are environmentally suitable for growing guayule—areas with low, nonfreezing temperatures and high daytime temperatures—salt and cold tolerance are not the limiting factors. How much, then, of the limited research resources should be invested in enhancing salt and cold tolerance at this stage of guayule development? Should cold tolerance

be as important a selection criterion as other factors such as selection for high-molecular-weight rubber? These and other questions must be answered when decisions are made regarding allocation of research dollars.

Setting priorities also means setting levels of funding for components of a research program. Extensive research experience has shown that to maintain a viable interdisciplinary research program, a minimal critical mass of scientific resources is needed. Thus, given the distribution of a comprehensive, balanced research and development program, the level of resources for a given component cannot be reduced below a minimum and still maintain a viable overall program. For example, if funding for a plant-breeding and genetics component of a program is decreased to the point that it is impossible to retain at least one fully supported scientist, it may mean that this component of the program cannot survive. Determining acceptable minimum levels of funding for various components of a program is a critical issue as these components may impact on one another and affect the viability of the entire program.

Some components of a research program may be conducted on an intermittent basis. For example, because the responses of cultivars to environmental factors such as water management follow general trends, new, detailed field experiments probably will not be required on a continuing basis. Periodically, the new cultivars will need to be evaluated and compared with older cultivars to establish new yield and response coefficients.

Plant breeding for increased rubber content and production should also involve an interdisciplinary approach so that the new lines will have increased tolerance to pathogens and insect pests (Chapter 8).

Priorities

Typically, available resources do not enable all research needs to be addressed at optimal levels simultaneously. Priorities need to be established based on the greatest need and the sequence of advancement needed to achieve the overall goal. For example, in 1983, scientists agreed that

a long-term guayule breeding program was needed. As projects were completed, resources that could be redirected were shifted toward the establishment of a viable genetics and plant breeding program. The breeding and genetics program had sound long- and short-term goals and objectives, and this research has made significant progress.

Resource Requirements

The level of resources required for a viable research and development program depends on the time planned to achieve a goal as long as minimum levels are maintained for components of the program as previously indicated. Some aspects may require special facilities. For example, processing technology may require additional pilot plants for testing new processing technologies or for processing shrub in a geographically different area.

Target/Client Groups and Needs

The research needs of new factors, such as involvement of the private sector or development of new uses for the guayule shrub, will need to be determined. New potential markets will need to be developed so that as guayule rubber production and guayule coproducts are increased, there will be a ready market for these products.

Implementation Strategy

A stable, long-term guayule research and development program will first require a commitment to the commercialization of guayule rubber production, followed by an agreement on a comprehensive, integrated research program. This may require an organized research and development workshop involving officials responsible for assuring that adequate supplies of natural

rubber are available in case of an emergency, administrators of research programs, and research scientists and engineers. The participants would be charged with arriving at a consensus regarding these issues before the end of the workshop.

Monitoring and Evaluation

Following the redirection of existing research or the implementation of new research, periodic monitoring and evaluation will be needed to assure that adequate progress is being made toward goals. When progress plateaus have been reached, resources will need to be redirected to other areas to fill information gaps or to increase the effort in promising new areas.

A Starting Point

The research needs previously identified by guayule researchers provide the best foundation for the future guayule research and development program. The first step will be to obtain a long-term commitment to the goal of commercializing guayule rubber production—developing a domestic natural rubber industry. The following is a concise statement of a guayule program for discussion purposes:

Program: Guayule Rubber Research and Development

Objective 1 - Develop improved guayule germplasm.

Approaches:

1.1 Develop new lines with higher rubber content and quality.

1.2 Increase basic understanding of guayule genetics.

1.3 Develop increased understanding of guayule molecular biology, biosynthesis, and bioregulation in relation to rubber production.

Objective 2 - Develop improved agronomic practices for guayule.

Approaches:

2.1 Improve seed production and handling technology.

2.2 Develop improved harvesting and shrub-handling technology.

2.3 Develop ecosystem simulation models that integrate all aspects of guayule growth in response to environmental variables.

Objective 3 - Develop improved guayule processing technology.

Approaches:

3.1 Refine rubber quality parameters and establish standards.

3.2 Develop improved, lower-cost processing technology.

Objective 4 - Develop new guayule rubber uses and markets.

Approaches:

4.1 Identify potential new uses and markets.

4.2 Assess comparative advantages of guayule rubber.

4.3 Assess potential national and international markets.

Objective 5 - Develop refined economic models of guayule rubber production.

Approaches:

5.1 Develop refined agronomic models and databases.

5.2 Develop improved harvesting and transportation models.

5.3 Develop improved rubber and resin processing models.

SUMMARY AND CONCLUSIONS

Guayule, a native plant to North America, is drought tolerant. Its drought-tolerant characteristics include a deep root system and the ability to go into partial dormancy when under water stress.

Guayule rubber formation occurs as deposits in plant cells while *Hevea* rubber occurs as

latex in plant ducts. This difference has major economic impacts on the competitiveness of rubber production and processing.

At the beginning of this century, natural stands of guayule provided the major source of raw material for natural rubber produced in the United States; these stands were depleted. Sustained production required establishing guayule as a commercial crop. Two major forces impacted this effort: 1) the need for a dependable source of natural rubber during World War II and 2) large increases in oil costs in the 1970s.

Guayule research conducted during the past 15 years has filled many gaps in the guayule database and has expanded our knowledge base. Detailed experiments and field trials have provided reliable production and performance data. These data, though limited, have squelched many speculative claims that had been made and propagated by promoters and speculators. The interdisciplinary research effort established national and international communication linkages.

The future guayule rubber development effort will require establishing new policies and national goals. Policy issues concern national security and more effective use of domestic resources. A national goal of establishing a reliable domestic source of natural rubber may be essential if guayule development is to continue. If a commercial guayule rubber industry is to be established, then a stable, well-balanced research and development program must be maintained.

Guayule research resources must be allocated proportionally to those areas that have the highest potential payoff in terms of increased production per unit area and production efficiency. Some components of the research program can be conducted intermittently. Others must be sustained continuously to make effective use of research resources.

The first step in developing an domestic natural rubber industry is obtaining a long-term commitment to this goal. A research program that will lead to this goal involves germplasm development, agronomic practices, processing technology, new rubber uses and markets, and refined economic models based on actual production and performance data.

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Index